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Getting at the truth about Dr Marshall

DR WALTER MARSHALL'S impending departure from his post as chief scientist at the UK Department of Energy (DEN), announced suddenly at the beginning of last week, raises certain obvious questions. Why? Why now? And what are the consequences? The matter is important. The storm accompanying the move has so far only amounted to a survivable squall rather than an intolerable hurricane for the minister who ordered it, Mr Anthony Wedgwood Benn. But it has rightly attracted more publicity than any similar move since the institution of chief scientist became a familiar part of the Whitehall scene. The reason is simple. It seems as though Marshall didn't fall; he was pushed—and almost certainly to make way for someone else.

The question of timing looks easy to answer, but isn't. True, Marshall had just completed the first round of reports on the exploitation of alternative energy sources; and important nuclear decisions are now coming up. Marshall had held two posts simultaneously—chief scientist at the DEN, for which he wasn't paid, and Deputy Chairman of the UK Atomic Energy Authority (UKAEA), with responsibility for its scientific and technical policy. So if he had to go from the former, this was the moment. As the blurb itself 'explained' at the time, Marshall would be resuming work in the UKAEA in view of those upcoming decisions and the UKAEA's 'significant role' in them.

It is not just that there is plenty of work still to be done in the non-nuclear field, in the execution of which Marshall is incidentally now a master, that has left people unsatisfied with this explanation. The various suggestions that Marshall was sacked, which Mr Benn has resented, have been based on the idea that when Marshall acted as Mr Benn's adviser rather than executor, he was 'pro-nuclear'; in other words, he was trying to push Mr Benn into taking early decisions on the choice of reactor issue and the fast breeder, and was generally giving unpalatable advice in an unpalatable manner.

If this argument has any credence at all, it demands refinement. For Marshall, formally speaking, was not Mr Benn's nuclear adviser. That was and is the statutory responsibility of the chairman of the UKAEA, Sir John Hill. Marshall would, of course, have given his view on nuclear issues if and when he was asked. And he had a good working relationship with Hill, his senior at the UKAEA, on responsibilities in this area. But if advice on the nuclear issues with which Mr Benn has become increasingly preoccupied is the reason for Marshall's departure, then *prima facie* it seems the wrong man is going.

Moreover, if the fact that Marshall is 'pro-nuclear' was now causing problems for Mr Benn, it had not caused him to worry in the past. It was Mr Benn who promoted Marshall 18 months ago to the Deputy Chairmanship of the UKAEA, even though Marshall was still to carry on being chief scientist. And it was Marshall who was reluctant to accept the promotion because of the conflict of interest, and

who only did so after asking the minister to consider it again and after receiving assurances that he was satisfied.

It is possible that Mr Benn now regards this conflict of interest, much commented on in the past, as intolerable. Certainly Marshall must have felt the need for his departure to be made public immediately for his own personal integrity. But though decisions are due, nothing specific appears to have happened in the past 18 months to make Mr Benn change his mind. Neither is he likely to be grooming Marshall for the UKAEA chairmanship when Sir John Hill's appointment comes up for renewal later this year. Marshall may be only 45 with 20 years of public life left in him, but Hill is 56 and has 10 left himself. Hill's handling of his public relations may have been shocking—indeed appalling on the choice of reactor issue—but there is no sign that he is on his way out.

That being the case, putting Marshall back so precipitately into the UKAEA looks more like a humiliation than anything else. But what could Marshall have done? His record is almost impeccable. He has galvanised the department into action in the field of research and development, producing acclaimed reports on R&D strategy, alternative energy sources and combined heat and power. He has stimulated vitally important work in energy conservation and offshore technology. He has helped Mr Benn to put a young department thoroughly on the map in a way that Mr Benn may not even now appreciate.

Not only that. The consequences of his departure reveal an important paradox surrounding the whole affair. The move is a depressing setback for all those engaged in work on non-nuclear energy, outside as well as inside the department. The loss of morale could prove costly, not least for Mr Benn. At the same time, though, there is little reason to doubt that the affair will *not* prove a setback on the nuclear side, the area in which the real controversies have been raging and on which Marshall would only have given his view in an individual capacity as Mr Benn's personal adviser.

Now this could well have proved a problem, as many people have suggested. Marshall had arrived at particular views. He would have advised that, on the choice of reactor issue, Britain should switch to the pressurised water reactor and export as many as possible; that, on the fast breeder, one big reactor should be built with all deliberate speed; and that, on reprocessing, it should be done for environmental reasons and, by keeping recovered plutonium where it could be controlled, as an anti-proliferation policy.

Some or all of this Mr Benn may have found unacceptable. But none of it seems to bear strongly on Marshall's departure, especially given that the UKAEA's position is more stridently 'pro-nuclear'. Only the manner in which such advice would have been given, and the mere fact that it was there impinging on decisions, seems directly relevant. Both of these appear to be strong possibilities.

Marshall and Benn are like chalk and cheese. Marshall



will endeavour to appraise a problem and give a considered answer whatever its implications. Asked to do things, he gets them done—and with the department's thinking complete, a 'doer' is what it has needed and still demands. He will be a loss in that respect alone. Mr Benn is different. He must be happy with Marshall's record in executing R&D. But he would have found Marshall irritating to have around with nuclear matters such a big preoccupation. As an ideas man and a political animal to the core, moreover, he needs advisers to brainstorm with who are well-versed politically. And he needs them full-time. Marshall was no intellectual dilettante, and wasn't full-time. Benn is no scientist. And he may not want to get things done.

This other point, that delays are costly, is also important; Mr Benn's advisers have long been urging that decisions be taken. On the other hand, however, the nuclear pause, as Mr Benn calls it, is a bind for bureaucrats, who need goals and the work goals provide—and the nuclear field is a huge chunk of the department's landscape. The industry, too, wants decisions. Now it may well be that in favouring delay for the sake of a public debate Mr Benn has encouraged confrontation, and sought only to ride the back of a good issue for his own personal advancement. But to attribute motives of personal political convenience to actions that have helped to put a complacent industry properly on its toes may be unkind.

Suppose, then, that there may be a good deal in what Mr Benn himself says about now wanting an adviser who can range over the full spectrum of energy matters full-time. If Marshall's nuclear and non-nuclear experience doesn't equip him to do just that, and if it isn't enough simply to speak of Marshall as having suddenly become an unacceptable irritant, there must be a problem above and beyond the fact that to appoint Marshall to a full-time post as adviser would be a rebuff for Sir John Hill. Indeed, not to have made Marshall full-time adviser with a brief ranging over all fields is again a humiliation.

What could this other reason have been? The suddenness of the announcement suggests that the decision was not thought out. But it had apparently been brewing for some time. So perhaps it is better to look at things from the other side. Although it may really be better to announce decisions like this than have them leak out, not announcing a replacement, and saying that an official is to leave 'as



Marshall: did he fall or was he pushed?

soon as possible', is unusual. A presumption has to be made that a replacement will be announced shortly.

That, however, is difficult to do by recruiting from outside civil service ranks, and certainly cannot be done quietly. It is thus more likely that the post will be filled by someone already available. The obvious place to look is among the other chief scientists. Apparently Mr Benn wants another chief scientist as his adviser. And the one who has the necessary experience and who might fit Mr Benn's requirements—more than any other—is Sir Hermann Bondi, chief scientist at the Ministry of Defence. No one else is in sight, and Bondi, having done six years in his present post and being a greater believer in a varied diet, might well find the move attractive.

If that happens, plenty of people would welcome it, not least various people at the Ministry of Defence itself. So one would wonder whether the whole operation was perhaps set up before Marshall's departure was announced. If so, Mr Benn's advocacy of open government, sincere as it is, looks thinner as a consequence. More importantly, his experiences with Monty Finnieston, Arthur Hawkins and now Walter Marshall suggest that he has a reputation for not being able to get on with his advisers. With such important decisions looming, that is the biggest worry of all. □

The concept of usefulness

Among other things, UK research councils are expected to support 'useful' research. In an article based on a lecture given at the Rutherford High Energy Laboratory, Sir Alec Merrison, Vice-Chancellor of Bristol University, comments

SCIENTISTS and society interact in many ways and the interaction is mediated by many agencies. The principal mediating agency in the UK is the government and in turn the government entrusts a great part of its mediating role to the research councils. Society makes demands of scientists and scientists make demands of society and in recent years these demands in both directions have become large.

In the past 15 or 20 years there has been a marked contrast between the mood of society in this country, very much determined by the sense of national failure, and the mood of science everywhere, which has been extremely buoyant. So government has looked to science (and technology) to be 'useful' and this was very much in the mind of the Labour government when it came to power in 1964 and again in the mind of the Tory government of 1970.

It is important to understand that the concept of use-

fulness is a decidedly limited one when applied to research, indeed it has to be applied with a good deal of taste. It is easy to identify certain research which will probably be useless in the economic sense, and just as easy to identify research which will certainly be useful. But these are limited areas and in between there is a vast body of research whose usefulness it is difficult, if not impossible, to quantify. Look at how much the world has been changed by the discovery 45 years ago of the neutron, and think how much it might be changed in the next 20 years or so by the discoveries of molecular and cell biology. But governments, and *a fortiori* ministers, do not last for 45 (or even 20) years and so, for this and other more compelling reasons, the time-scale is all-important in judging usefulness.

There have been two major revolutions in the past 15 years in the way research councils work: the one set in motion by the Trend Report of 1963 and the one flowing from the White Paper *A Framework for Government Research and Development* in 1972. It was on the Trend Report and the consequent Science and Technology Act of 1965 that our present framework of five research councils has been based. But one of Trend's recommendations, pro-

posing the creation of an Industrial Research and Development Association, was inflated by the then new Labour Government into the now defunct Ministry of Technology. The aim of the 1965 Act was to bring some rationalisation and coherence to the civil research sponsored by public funds, and this it certainly did. But it was a recurrent theme through the 1960s that the research councils were not paying sufficient heed to the nation's needs, insofar as these were expressed by the government's spending departments. In 1970 this found expression in a bid by the Ministry of Agriculture to take over the Agricultural Research Council. This matter was examined by a committee of civil servants, chaired by Mr S. P. Osmond, who recommended that it should happen. Before agreeing, the then Secretary of State for Education and Science asked the Council for Scientific Policy, whose remit it was to advise her on all matters concerning the research councils, to advise her "on the most effective arrangements for organising and supporting pure and applied scientific research and postgraduate training". A small working party under the CSP Chairman, Sir Frederick Dainton, reported to her in May 1971 that its view was that the research councils should be left largely untouched but that the CSP itself should be reformed so that account could be taken of the views on policy held by government departments.

Just after this report was submitted, Lord Rothschild was appointed as first head of the Central Policy Review Staff and the government asked him to review the management of the whole of government research and development including that falling within the purview of the research councils. Lord Rothschild enunciated his now famous 'customer-contractor principle' which was to be applied to all research and development other than basic research. He recommended that in the case of three of the five research councils (Agriculture, Medicine and Natural Environment) there should be a large transfer of their funds to those departments which had an interest in their work. The departments would then use the research councils as their contractors to carry out work which they commissioned.

In the White Paper of July 1972 the government decided that the CSP should indeed be reformed, largely along the lines suggested by the Dainton Committee, and thus it became the Advisory Board for the Research Councils. They accepted too a modified form of Rothschild's customer-contractor principle and his proposal to transfer funds (roughly 50% from the ARC, 30% from NERC and 25% from the MRC). It is perhaps unnecessary to say that rather elaborate machinery has inevitably had to be set up to supervise the allocation of this transferred money.

There are three questions which can be asked in the case of each of these major reforms. Has it made any difference? Has it done society any good? Has it done scientists any good? Now is certainly the right time to ask these questions, since it was the Tory Government's intention when they published their 1972 White Paper that at least five years should elapse before any further substantial change should be contemplated. But in answering them it must be borne in mind that they are essentially questions which are not only difficult to answer, if indeed they can be answered, but also that any answers which can be offered must contain a good deal of subjective judgment.

Both reforms have certainly made a difference and I would imagine that this at least is an answer which would arouse little dissent. So far as the 1965 Act is concerned my own view is that both society and scientists have benefited. A reasonably flexible system has been created which at the same time is sufficiently cohesive that the 'Science Vote' is a good way to control the funds at the disposal of the research councils (not that they are ever enough!).

We are still rather close to the 1972 reforms, but five

years later it is difficult to see that they have had any really substantial effect, and such effect that they have had seems to have been for the worse. They have certainly not done the scientists any good, but neither have they done them very much harm (with the possible exception of a good deal of nervousness on the part of scientists working in the more fundamental areas).

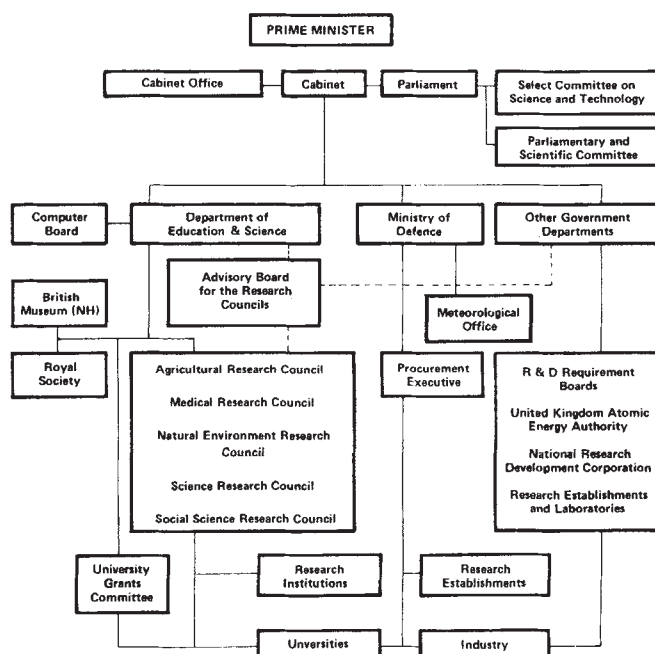
What has unquestionably happened is that an unnecessarily elaborate machine has been set up to supervise the spending of the money transferred from the research councils to government departments. This is certainly a loss to both scientists and society and should be reformed. It is wasteful of people's time and is yet another piece of inefficient bureaucracy.

Equally unquestionably, what has not happened is a re-direction of research to any conspicuous degree. One can ascribe this to the incorrigible waywardness of scientists or to the fact that they were not after all quite so insensitive to the needs of society as some people believed. My own view would be that it is largely the latter. At any rate, society is on balance the loser because of the 1972 reforms.

One of the weaknesses of the transferred money system is that once the control of a budget gets into the hands of a spending department then that budget will be at the hazard of all sorts of departmental pressures, few of which will have anything to do with whether the research which might be funded is good or bad. I can hardly believe that last year, when the DHSS decided not to spend nearly a million pounds of its transferred money, it took the decision simply on the grounds that it had come to the conclusion that it was not getting value for money.

So it is a good time to examine carefully the benefits and disadvantages of this way of government departments and research councils working together and, if it really is more trouble than it is worth, to scrap it. It would be good to look too at the whole conspectus of the government's support of 'useful' research and see whether the balance is right. Are we spending the right relative sums on aeronautical research and civil engineering, for example? But if any review is to be undertaken, on a narrow or broad front, I very much hope the reviewers bear in mind the fragile nature of the concept of usefulness in this field.

UK Research Councils: Where they fit



OTA's stormy ride

Colin Norman in Washington examines the history and prospects of the Office of Technology Assessment

A SERIES of political upheavals has badly shaken Congress's Office of Technology Assessment (OTA) during the past few weeks, prompting speculation about the future of the fledgling agency and providing ample grist for the rumour mills of Washington's small corps of science policy watchers.

It began on 20 May, when Emilio Q. Daddario, OTA's founder and first Director, announced that he would resign to take an as yet unspecified new job. In his letter of resignation, Daddario said that he felt OTA was firmly established and noted that he had long expressed a desire to leave as soon as the office had reached that point.

Less than a week later Representative Marjorie Holt, a conservative Republican from Maryland, announced her resignation from OTA's Congressional Governing Board. The reason for her departure, she said loudly, was that OTA had been "taken over" by Senator Edward Kennedy, chairman of the board, and she felt that she could no longer make a contribution to OTA's affairs. Her grievances were promptly given a full airing by William Safire, a former speechwriter for Richard Nixon, who wrote a column in the *New York Times* charging that Kennedy had removed Daddario in order to install his own aide, Ellis Mottur, as director.

Then a third blow fell last week. The House of Representatives, acting on a recommendation of its Appropriations Committee, slashed the budget request for OTA next year from \$9 million to \$7.4 million and decreed that the office's staff level should be reduced to 100, thirty less than now.

This political crossfire is the culmination of a number of tensions which have been building up in OTA ever since it came into being three and a half years ago. The bloodletting that has resulted may ultimately be beneficial for the agency, but much will depend on who succeeds Daddario.

Difficult gestation

OTA's birth followed a long and difficult gestation period which began in the late 1960s. Congress was then beginning to grapple with a series of complex technological issues, such as whether the United States should proceed with construction of an SST and whether an anti-ballistic missile system should be deployed. The debates starkly underlined the fact that Congress

lacked the resources to match the technical expertise of the executive branch on such complex matters. At that time Daddario was a Congressman from Connecticut and he held a key position as chairman of the science subcommittee of the former Committee on Science and Astronautics.

After holding a number of hearings with his subcommittee in the late 1960s, Daddario sponsored legislation to create an agency of the Congress to assist in the evaluation of technological matters and to provide an early warning system to pinpoint potential side effects and long term implications of projects and policies involving science and technology. Congress finally passed a bill establishing OTA in October 1972, though by that time Daddario had left Congress to run (unsuccessfully) for the governorship of Connecticut. Kennedy played a leading role in shepherding the bill through the Senate.

The bill specified that OTA would be run by a 13-member board, consisting of 6 Senators, 6 members of the House and the director of OTA. Republicans and Democrats would be equally represented on the board. In addition, the legislation established an independent advisory committee to provide policy advice to the board.

The legislation also specified that the chairmanship of the board should alternate every two years between Senators and House members. Kennedy was elected the first chairman. He was followed by Olin Teague, chairman of the House Committee on Science and Technology, who served during 1975 and 1976, and Kennedy resumed the chairmanship earlier this year. Daddario, meanwhile, was appointed director in 1973 for a six-year term.

OTA represented a major innovation in the Congressional process. It was designed to provide Congress with long-range analysis, act as a counterweight to the technological expertise of the executive branch, and function as an early warning system. It is very much a creature of the Congress, having to respond to the concerns of legislators and provide analyses of issues on a timescale which can effect the passage of particular bills. One OTA staff member last week likened OTA's establishment to an organ transplant: "We grafted it into Congress and waited to see if it would take", he said.

The graft took slowly, and there



Daddario: just changing jobs?

have been occasional signs of rejection. First, it took a long time to get the initial studies under way because Congress was slow in appropriating money for OTA. There were also a number of internal problems in securing office space and assembling staff. But OTA began to crank out a huge volume of material. Some 46 studies have now been completed, and 34 more are in the works. Topics have ranged over a broad spectrum, including the bioequivalency of supposedly identical prescription drugs, policies to curb nuclear proliferation, the potential for small scale solar technology (see page 6), and a broad assessment of the impacts of offshore oil and gas production. OTA has also produced a number of critiques of specific administration policies, such as energy plans published by the Energy Research and Development Administration (ERDA), President Carter's energy policy, and the research and development strategy of the Environmental Protection Agency.

Though arrangements differ from one study to another, the general procedure is to establish an advisory committee of outside experts to help plan and monitor each project. The advisory panels are usually deliberately chosen to reflect diverse points of view, frequently containing members of public interest groups and businessmen. The studies themselves are usually carried out by OTA staff, sometimes with the help of outside contractors, under the guidance of the advisory panels and with overall management provided by a programme director.

Important criticisms

But OTA has run into a number of important criticisms during its short lifetime, and there have been occasional signs that Congress may reject this strange transplant. The grumbles have, however, concerned OTA's style of operation rather than the quality of its reports and analyses.

The first complaints came from OTA's advisory council, which grumbled that it was not being sufficiently consulted on matters of policy. Then, in June last year, the House Commission on Information and Facilities, an obscure Congressional unit headed by Representative Jack Brooks, charged that OTA's internal management was in a mess and that there was general confusion about the agency's mission. That blast was followed by criticism from Harold Brown, now Carter's Secretary of Defense, who resigned last July as chairman of OTA's advisory council and claimed in his letter of resignation that the office had become bogged down in responding to requests for relatively trivial policy studies to the detriment of its primary mission of providing an early warning system. Then last October, a House/Senate conference committee recommended that OTA's budget should be cut and suggested that the fledgling agency had yet to prove its worth to Congress. Finally, from outside OTA have come sporadic complaints that the office has been too cautious in its approach and that it has not really been performing technology assessment.

Asked to comment on some of those criticisms in an interview with *Nature* before he left office, Daddario acknowledged that, "Sure we have had our problems, but every institution in this town has had its problems". He pointed out that most of the criticism was directed at bureaucratic arrangements when OTA was still feeling its way and evolving its style of operation. It has taken three years to develop a sound process to handle the studies, Daddario said, and suggested that it could take at least a decade before the office is functioning smoothly with the full support of the Congress. Daddario noted that none of the criticism has been directed toward the quality or credibility of OTA's studies and it is that, after all, which will ultimately

determine the office's influence.

It is indeed possible to point to a number of OTA analyses which had an impact on policy. The critiques of energy policy, for example, suggested that ERDA was paying too little attention to conservation, and the policy was subsequently changed. ERDA Administrator Robert Seamans acknowledged in a press conference early last year that OTA's analyses had played a significant role in shifting funds into conservation programmes in ERDA. The study on offshore oil and gas production has attracted attention and is praised for raising public awareness of many of the central issues involved in the Administration's plans. The drug bioequivalence study, which was the first published by OTA, played a major role in shaping federal policy for drug purchasing under government-financed health care schemes.

Though OTA staffers are quick to note such examples of the office's influence, there has so far been no coherent review of the quality of OTA's reports or the use to which they are put. Two such studies are, however, being planned. The first will be conducted this autumn by the House Committee on Science and Technology, and the second will be carried out by OTA's own advisory council. The latter study was initiated earlier this year by OTA's Congressional board, largely at the urging of Senator Kennedy. And that brings us back to the latest round of political upheavals at OTA.

Kennedy allegations

Allegations that Kennedy planned to take control of OTA were raised when the office was first established and Kennedy was elected chairman of its board. An article in the *Wall Street Journal* in 1973, for example, charged that Kennedy would use OTA as the springboard for his 1974 Presidential election campaign, and in the same vein Safire's recent column in the *New York Times* suggested that Kennedy has little influence on the Carter Administration so he is quietly taking control of OTA to build up his own power base.

Few people deny that Kennedy has a strong influence on OTA affairs. His supporters claim, however, that his influence derives from the fact that he is the most active and interested member of the board. His detractors claim that his influence derives from the fact that some of his own staff members work for OTA and that the chairman of OTA's advisory council, Jerome Weisner, is an old political ally of Kennedy's. It should be noted, however, that Kennedy is not alone in having his own staff working for OTA.

Other Senate members of the board have at least one staff liaison with OTA, a fact which has drawn criticism from some other OTA staffers because the political appointees have divided loyalties.

Mrs Holt told *Nature* last week that she resigned from the board because she felt that it "was obvious that we were having no opportunity to make any input at all". On three recent issues Holt was outvoted on the board by Kennedy and his supporters. In each case, she was supported by Teague and the other two House Republicans. The first issue arose when the board refused to reappoint a Texas Instruments executive, Fred Bucy, to a second term on the advisory council. Kennedy opposed the reappointment because Bucy had attended too few meetings in his first term; Holt claimed that Kennedy simply didn't like Bucy's stand on some issues. Second, the board sanctioned a quick assessment of the data which led to the proposed ban on saccharin over Holt's objection that such a study wouldn't add anything to the debate. And third, Holt unsuccessfully resisted a move to have the advisory council conduct a study of OTA's operations.

Underlying the dispute is a significant political gulf between Holt, a conservative Republican, and Kennedy, a prominent liberal Democrat. OTA, in short, has become a battleground for partisan politics.

As for Safire's allegation that Kennedy had engineered Daddario's resignation to pave the way for installing his own candidate in the director's chair, Daddario insisted that his resignation stemmed from nothing more than a desire to change jobs. He said that he had originally planned to stay for only three years but stayed longer to see the solar study and the critique of Carter's energy policy through.

Whether or not these upheavals will leave lasting scars depends largely on who is chosen to succeed Daddario. Kennedy is taking scrupulous care to avoid suspicion that he is trying to influence the selection process. He has asked for nominations from scores of organisations, the advisory council will screen the candidates and the board will make the final selection. So far, the only announced candidate is Daniel V. Desimone, who has been deputy director for the past three and a half years. Ellis Mottur, who now heads a major OTA programme, has been nominated by several groups, but he told *Nature* that he has asked the council not to consider him because his close ties with Kennedy would be sure to encourage Kennedy's opponents.

The next few months are clearly going to be critical for OTA. □



Kennedy: how much influence?

USA

Suit to measure

David Davies details a recent US court decision to settle a libel action involving scientists

A RECENT decision in the United States Courts of Appeals has overturned a libel judgment against the *New York Times* and an officer of the National Audubon Society. A New York jury had earlier ruled as libellous accusations in the *Times* that certain named scientists were paid by the pesticide industry to lie by misinterpreting bird-count figures; these figures might have been relevant to the question of DDT's effect on animal life. But the appeal court ruled that the reporting was privileged under the First Amendment.

The dispute arose out of the Audubon Society's report in *American Birds* on its 1971 Christmas Count. The editor, Robert Arbib, wrote a foreword warning that segments of the pesticide industry and certain paid "scientist-spokesmen" were misusing bird counts to prove that bird life is thriving. He continued that apparent increases had in most cases nothing to do with real population dynamics. They were the result of ever increasing numbers of birders, better access, better knowledge and more sophisticated iden-

tification. He concluded: "What we are seeing is a result of *not more birds, but more birders*. Any time you hear a 'scientist' say the opposite, you are in the presence of someone who is being paid to lie, or is parroting something he knows little about".

A *Times* reporter, John Devlin, saw the article and asked Arbib to name some "paid liars". Arbib turned to Roland Clement, the society's Vice-President, for help; Clement provided a list of "consistent misinterpreters" of data, adding however, "We don't have any knowledge of anyone being a paid liar". Whether this qualification was actually passed by Arbib to Devlin was strongly disputed at the original trial.

Devlin next tried to get comments from the five named—J. Gordon Edwards, Thomas Jukes, Robert White-Stevens, Donald Spencer and Norman Borlaug. He reached White-Stevens, Jukes and Spencer, who hotly denied the charges—White-Stevens and Jukes sent copies of their publications. Devlin then wrote his story which appeared in the *Times* in August, 1972. It quoted the Arbib foreword extensively and added that Arbib had said in an interview that the men under attack included the five named. Devlin wrote that those he had reached "ridiculed

the accusations as 'emotional', 'hysterical' and unfounded".

There was understandable alarm at the Audubon Society with the *Times* story, and Clement sent round an internal memo that "Arbib was trapped into listing people I have never called liars, though they may be". Clement also dispatched a letter to the *Times* over Arbib's signature saying "it is not simply that more birds are reported because there are more birders, but that we have more birders who are also better birders. Nor do we like to call people liars, but those who have most consistently misused our data [the five scientists were named] . . . have had time to learn from our patient explanations . . .".

Jukes also wrote to the *Times*, claiming "we have reported these figures in birds per bird watcher". Neither letter was published. Jukes, White-Stevens and Edwards sued for libel and received a total of more than \$60,000 in damages against the *Times* and Clement. Arbib was exonerated.

Libel actions by public figures in the United States (and the three plaintiffs were so ruled) can only succeed if it is shown that the libel was motivated by malice—that is, with knowledge that the charges were false, or with reckless disregard of whether they were false or not. The lower court apparently had no doubt that the charges were false and that insufficient care had gone

OTA's bright solar report

GIVEN relatively modest federal support, small solar energy devices could play a significant role in the United States energy picture within a decade, and their potential impact in many developing countries is even greater, according to a massive study published this week by the Congressional Office of Technology Assessment (OTA). Perhaps the most detailed report on solar technologies yet produced, OTA's 1,330-page document is likely to have a major impact on federal policy, coming as it does in the midst of Congressional debate on President Carter's energy proposals.

The report's central message is that the key factor governing the speed with which solar technologies capture the market is the relative cost of conventional energy resources. No major technological breakthroughs are needed for widespread use of solar heaters, and other technologies are also well advanced technically, the report says.

At present, solar heaters can compete economically with electrical heating systems in a limited number of situations, when the capital costs plus the lifetime fuel costs are taken into consideration. But the OTA report suggests that if electricity prices climb by a relatively modest 40% over the next quarter

century, solar heaters will be economically attractive in most areas of the country by 1985. Moreover, if 20% of the capital cost of solar heaters could be offset against income tax, they would be competitive much sooner. As for non-electrical heating systems, the OTA report suggests that solar heaters will be cheaper than systems using synthetic gas, but will probably be more expensive than coal-fired systems at least during the next two decades.

The study is also optimistic about the potential for more exotic technologies, such as the direct conversion of solar energy to electricity by photovoltaic cells. Though such devices are now inordinately expensive, the OTA study suggests that the costs could be brought as low as 5–15 cents per kilowatt hour within a decade. That would still be well above present electricity prices, but it may be in line with electricity prices from new generators in the late 1980s. The key, again, is the extent of incentives such as tax breaks for both purchasers and manufacturers of photovoltaic equipment, but research breakthroughs will also be required to bring down the price of photovoltaic cells.

The OTA report breaks new ground in assessing the potential impact of a large solar industry on the labour

market, and its findings are likely to enhance the political prospect for solar power. Widespread production and use of small solar units would create employment in such industries as sheet metalworking and light manufacturing which are now experiencing serious unemployment problems, OTA concluded.

Since the rate of introduction of solar technologies depends critically on the relative costs of competing energy sources, solar power is likely to be particularly attractive in countries where fuel costs are high and labour costs are low. Those conditions apply in most developing countries, and thus the OTA study concludes that the potential for solar power in the third world is great. Widespread use of solar technologies would carry the added advantage of reducing international competition for dwindling fossil fuels, and it would also reduce the incentives for developing countries to purchase nuclear plants, the report notes. It is thus in the United States' political and commercial interest to foster an international market for solar technology, the report points out, and the best way to stimulate such a market is for the United States to launch its own domestic solar energy programme.

Colin Norman

into investigating the allegations after the scientists had claimed they were baseless.

The appeal court in no way set aside the findings of the lower court that the accusations were defamatory. Indeed, it used the words "completely foundationless accusation of venality" to describe the charges. What it did rule, however, was that Devlin was insufficiently warned of the falsity of the charges by the scientists' responses, and so could not be accused of malice: "Mere denials, however vehement, are so commonplace . . . that they hardly alert the conscientious reporter to the likelihood of error". Nor, the court ruled, did the "largely irrelevant" mass of materials supplied provide a basis for warning of the falsity of the charges. There was not a "shred of evidence" that Devlin entertained serious doubts concerning the charges.

Further, the court held that even if Devlin had had such doubts, "We do not believe the press may be required under the First Amendment to suppress newsworthy statements merely because it has serious doubts regarding their truth". The public interest in full information required freedom to report charges; accordingly Devlin's article was held to be an "exemplar of fair and dispassionate reporting". Clement was also cleared because it was held that he had not known the *Times* would portray the five as paid liars when he supplied the list to Arbib.

The ways of the law sometimes puzzle scientists and perhaps the most puzzling aspect of this case is how a scientist, told he is to be called a paid liar, can adequately rebut the charge. The court ruled that simply calling the charges "emotional", "hysterical" and unfounded wouldn't even alert a reporter to falsity. Nor would sending him the scientist's stock-in-trade reprints, apparently do. It isn't clear that the appeals court looked at what was sent to the reporter, but they dubbed it "completely irrelevant to the libellous accusations".

It is also noteworthy that the one scientific issue got completely lost in the case—whether the bird statistics establish anything at all. Arbib's foreword claimed that bigger counts per birder arose from better techniques, but then implied that paid liars would simply fail to divide by the number of birders. Devlin did not even mention the question of improved techniques, implying much more strongly that certain scientists don't divide. But the scientists claim that they do and that the figures are still up. The question of better reporting *versus* bigger populations to explain these rises—the nub of the difference between the sides—was hardly advanced at all by this bloody court battle. □

ASBESTOS

Amplified evidence

Britain's Advisory Committee on Asbestos held hearings in London last week. Judy Redfearn reports

THAT there is a risk to health from asbestos cannot be denied; but among the general public and in the asbestos industry itself, there are conflicting views on how acceptable that risk is. In March 1976, the UK Health and Safety Commission set up the Advisory Committee on Asbestos to review the risk to different sectors of the public and to come up with recommendations on how asbestos should be used, if indeed it should continue to be used at all. Last week, the committee held three days of public hearings in London when it questioned some of those who had submitted evidence for its consideration.

At the heart of the debate was the acceptability of the asbestos dust levels which were recommended by the committee in an interim statement published in January. Occupational exposure to asbestos dust, that statement said, should be as low as possible, but should not exceed 0.2 fibres per cc when measured over a ten-minute period for crocidolite (blue asbestos, thought to be the most dangerous sort), and 2 fibres per cc averaged over a four-hour period for all other types of asbestos. But the safety of these levels depends on how accurately the incidence of asbestos-related disease can be predicted. As with other diseases caused by long term exposure to certain factors, such as the incidence of lung cancer in smokers, this is very difficult to assess.

The committee's recommended exposure levels were based on the results of a study carried out in 1966 by the British Occupational Hygiene Society (BOHS) on workers in an asbestos factory in Rochdale. The study concluded that an asbestos worker would have a 1% chance of developing an asbestos-related symptom if exposed to 2 fibres per cc for 50 years. A subsequent follow up study at the same factory, however, has come up with some different results.

Dr Julian Peto, of the Department of Health and Social Security's Cancer Epidemiology Unit at Oxford, was responsible for the later study. He told the committee that by the end of 1974 deaths from asbestos-related disease in those exposed for 25 years or more exceeded the total number of expected deaths by 30%. In 1966, the BOHS found nothing unexpected in the death

rate. Peto's study also suggested that 5–10% of workers exposed for 50 years to concentrations of 1–2 fibres per cc of asbestos would be likely to die of asbestos-related disease. The discrepancy between the end result in the two studies, Peto suggested, could be due to the cumulative effect of asbestos exposure between 1966 and 1974 and the different way of sampling asbestos workers: Peto's study included all men who had been exposed to asbestos for 10 years or more between 1933 and 1972, whereas the BOHS study only included those still in employment there in 1966.

Furthermore, Peto told of evidence that chrysotile (white asbestos) could be as potent as crocidolite in causing asbestosis and pleural mesothelioma, an inoperable cancer of the membrane surrounding the lung. All in all he felt there was strong evidence that the present 2 fibres per cc level is inadequate but that there should be more research, especially into factors such as the effectiveness of pulmonary clearance below 2 fibres per cc.

But concern does not only centre on workers in asbestos factories. Asbestos is so widely used that most people at some time will have been exposed to it. Pressure is increasing to ban certain applications of asbestos such as asbestos paints and sprays and domestic use in general. The British Society for Social Responsibility in Science (BSSRS), using its evidence that cases of asbestosis and mesothelioma have resulted from very short exposures to asbestos, is calling for a total ban on its use; it suggests that in the meantime personal protection for asbestos workers should be improved and safe substitutes should be introduced as soon as they are developed. But even a far more stringent standard poses problems. The recommendation from the Trades Union Congress of a 0.2 fibres per cc standard was thought technically impossible to achieve for most manufacturing processes, especially the dry processes which create a lot of dust and which are found in the asbestos textile industry.

The committee's task now is to digest the evidence gathered at the hearings and to call others forward to explain their case. No one yet knows when it will produce its report laying down recommendations for the future use of asbestos. There are provisions, however, for another interim report to be published if the evidence suggests that speedy changes are needed to the present ways of dealing with asbestos. □

CZECHOSLOVAKIA

In a sorry state

Vera Rich reports on recent evidence of harassment of dissident Czechoslovak scientists

CZECHOSLOVAK scientists whose "moral and political requirements" fail to meet the official standard are not permitted to publish their scientific work. This was revealed by a 270-page White Paper on Czechoslovakia published last week the International Committee for the Support of Charter 77.

The Order was first made known in a letter from the Czechoslovak Ministry of Education to the Charles University, Prague, in May 1972, and in spite of protests has never been revoked. Since recent government moves against the Charter 77 movements include widespread dismissals of known signatories, the order on publication has taken on renewed importance.

One man who visited London to launch the White Paper, Dr Zdenek Mlynar, was dismissed from the Entomological Department of the National Museum for being a member by the International Committee for the campaign known as Charter 77. Now in exile, he says that with the new waves of dismissals of the known Chartists from the Party and from professional employment, "Prague today is full of highly educated men sweeping the streets and stoking boilers".

These dismissals differ in one respect

from those of the post-Dubcek repressions of 1970. Then it was primarily the social sciences which were hit—offending natural scientists were demoted but normally remained in their field of research. Today, however, the campaign against the Chartists makes no distinction between natural or social scientists; all are equally liable to dismissal, under the regulations on political attitudes.

These regulations, quoted in the white paper, actually antedate the Charter. The Directives of the Secretary General of the Academy of Sciences of July 1976 noted that "All creative and economic-technical employees shall have to be qualified in terms of political maturity . . ."; and all leading "scientific workers" of the Academy were requested to complete a questionnaire concerning their political activities and Party status, and also that of their close relatives.

Following the appearance of the Charter, there was a wave of summary dismissals of known Chartists, including Dr Mlynar himself, from academic posts without the customary period of notice; it was "not possible" for the Chartists to remain during the normal termination period "without jeopardising the orderly fulfilment of the organisation's tasks." Under the 1972 regulations on publication, scientists thus dismissed not only lose their posts; they are unable to publish work in hand, or undertake such fringe activities as translation.

When the restrictions first became known, Dr Frantisek Janouch, in a letter of protest to the Minister of Education, Josef Havlin, pointed out that they would inhibit the dissemination of "valuable information on new findings" solely because their authors' "moral-political profile does not comply with a certain set of curious criteria which have never been publicly announced". In the case of the Chartists, the criteria are even more curious because the Charter and its subsequent documents make no demands which are not already stipulated either in the Czech constitution or in the international human rights agreements to which Czechoslovakia is a signatory.

Whatever the criteria, however, the dismissal of scientists and the ban on publication are a considerable threat to Czechoslovak science. It is already difficult, said Dr Mlynar, to function adequately as a scientist in Czechoslovakia: personal international contacts are now only 10% of what they were in 1968; foreign currency restrictions have brought about a general drop in the standard of equipment and in the accessibility of foreign journals.

Now there is also a threat to the next generation of scientists. A large collection of documents in the white paper presents unequivocal evidence that parents' political attitudes are a prime factor in deciding university admissions. Again, this trend antedates the Charter; Document 4 of the Chartist movement (23 January 1977) specifically calls for an independent inquiry into this practice. This demand has not been met. □

WEST GERMANY

Saving it

The West German government has plans to encourage energy saving. Werner Gries explains how

CONDITIONED by the oil crisis the Federal Republic of Germany has now moved energy-saving to the foreground of its energy policy. It has put special emphasis on reducing energy losses in electricity generation, saving energy in heating supply, and saving energy used by traffic.

On the domestic front low interest loans have been allocated to finance energy-saving measures. Definitive regulations about heat insulation for housing construction have been laid down in energy saving legislation and new buildings will have to be better

insulated in future. Firing installations in residential buildings will also have to be made more efficient. This will increase the cost of building houses by approximately 3%.

Industry is to be granted 7.5% of its expenditure on energy-saving investments and special investment credits are already available. In the public sector energy-saving measures are being considered in building plans and public education in energy saving is being intensified. In research, prototype projects for the efficient use of energy are being supported directly by the Research Ministry; over the past three years about DM500 million have been made available for this purpose.

The government is at present working out a detailed programme. At the heart of further domestic measures are

investments to improve heat insulation and installations for heating supply. Heat pumps and solar collectors are also to be promoted through state allowances. The tenancy law is being altered to encourage energy saving. The government also wants to modify electricity tariffs and possibly make it a duty to mark the energy consumption on household appliances.

The focus in industry is on the coupling of electricity and heat production. An investment of DM2,400 million is to be spent on district heating up to 1980. Thought is also being given to taxing cars with diesel engines less than those with petrol engines and new driving systems (methanol cars and electric cars for example) are being encouraged through tax incentives. Most of the government measures, however, will only complement private initiatives and interventions will be limited. □

SWEDEN

● The National Environment Protection Board has proposed to the government that, from 1 January 1979, no aerosol pack containing halocarbons as a propellant should be made in Sweden or imported without permission from the Board's Product Control Division.

The Swedish initiative follows American research into aerosol packs and their effect on the ozone layer—an area in which the Swedes have not done any independent research. The present proposal was stimulated by last May's joint recommendation by America's Food and Drug Administration, Environmental Protection Agency and Consumer Product Safety Commission that would forbid the manufacture of halocarbons or of sprays containing them and the sale of both by April 1979.

The American recommendation exempts aerosols for certain essential uses, mainly medical (sprays for asthmatics, for example), and envisages other uses deemed essential in the future being added to the list of exemptions. The Product Control Division chemist who wrote the Swedish proposal, Ingrid Jedvall, sees much the same sort of situation emerging in Sweden, with certain aerosols being given permission to be sold. As an administrator she prefers her proposal to the American one because if all decisions about particular aerosols are taken within the division, no publication and updating of exemptions will be necessary. It could also be argued that the public should be informed which aerosols are being sold at any time. Critics will no doubt label the proposal as typical of a Swedish trend, which they see as government by bureaucrats whose tenure does not depend on responsibility to the people.

Sweden does not manufacture halocarbons, and uses less than 1% of the world's consumption. In 1975, the country used about 5,200 tons of halocarbons, but this amount has decreased to the extent that the 1977 total is expected to be only about 1,200 tons. The 1975 amount was used in the following proportions: cosmetics 65%, household products 6%, medical and veterinary 3%, colour, waxes and polishers 9%, insecticides 4%, products used in industry 12%, and other uses 1%. In the same year, 29 million packs were sold, an average of about 3.5 for every inhabitant.

The decreased use of halocarbons is partly due to changes in production introduced successively since 1973 by the Aerosol Packing Co., which sells

75% of aerosol cans in Sweden. It has changed from halocarbons to propane and butane and, as a company spokesman pointed out, will not be at all dismayed if halocarbons are regulated. To offset the fire risk present during manufacture under the new régime, the company has computerised the system so that the cans are monitored and the whole system can shut off automatically if concentrations of the gases reach the danger zone.

● Sweden's first wind power station was recently opened on the coast north of Stockholm, at Älvkarleby. Its 18-metre-wide vane, which rotates about an axis perched on top of a tower 25 metres high, generates between 50 and 63 kilowatts. Ungainly though it looks, its computer—which automatically adjusts the angle of the vane to the wind—should provide the state Committee for Energy Research with useful data about the way its design stands up to stress and vibrations under different wind conditions. It is, says one committee member, Sven Hugosson, a sort of laboratory bench unit whose performance will determine whether it will be worth building others of the same type. The first evaluations of the way it is going will be made this summer.

Whatever the results, they will not influence the next phase of the Committee's wind programme, due to begin in August. Three full-size wind power stations, each generating 2 megawatts but differing in design, will be located in various parts of the country with the help of a survey done by the Meteorological Institute describing wind conditions around the coasts. Their performances will determine which designs will be built in future. At the opening of the Älvkarleby station the Energy Minister, Olof Johansson, looked forward to the turn of the century, when he envisaged wind energy providing between 4,000 and 10,000 megawatts.

Sven Hugosson says he does not doubt the technical feasibility of wind power for Sweden, but that very little money has so far been appropriated for experimentation. He does not think it could replace any other source of energy, but is certain it could play a part—especially in conjunction with hydro-power—in providing total energy needs. It does have, however, some surprising hazards: chunks of ice may be flung off the vane in winter and scattered like mini-missiles.

Wendy Barnaby

IN BRIEF

JET: still waiting

Some intensive lobbying is expected throughout Europe over the next couple of weeks in the run-up to the EEC Council of Foreign Ministers in Brussels on 25–26 June, when a decision on the siting of the Joint European Torus (JET) fusion project is now due. The subject came up at the meeting of European heads of government in London last week. Reports of a West German effort to secure a greater UK contribution to the Community budget in return for the project going to Culham instead of Garching were not in the event borne out. The leaders failed to agree and the matter was passed over to the Foreign Ministers for them to decide in a make-or-break meeting.

At a press conference after the meeting, the UK Prime Minister, James Callaghan, said he doubted whether any individual country could carry on on its own if a solution satisfactory to everybody wasn't reached. But with such an excellent team at Culham, he said, "It would be my desire to see whether we could work out bilateral, trilateral, quadrilateral or multilateral agreements with any group of countries that will keep the project in Europe, even if it wasn't a European project in the technical sense".

World climate conference

At its 29th session in Geneva last month the executive committee of the World Meteorological Organisation (WMO) decided to convene in 1979 what it calls a high-level scientific and technical conference, called the World Climate Conference, gathering together meteorologists and experts on the effects of climate on economies.

The timetable of the first global experiment of the Global Atmospheric Research Programme (GARP) was approved. Gathering of atmospheric and oceanographic data for research will be planned this year and observations will be carried out from December 1978 to November 1979, especially during January–February and May–June 1979. The aim of the experiment will be to improve meteorological forecasts and to obtain a better understanding of climate.

Energy balance urged

Governmental machinery for striking the right balance between different energy sources, bearing in mind not only availability but the differing community and environmental costs, is "entirely lacking", says Sir Peter Kent in a letter to *The Times* this week. Sir Peter, chairman of the UK Natural

Environment Research Council (NERC), would like to see some inter-departmental organisation established—perhaps within the Cabinet Office—"to demonstrate that the energy problem is regarded as transcending departmental boundaries". Factual data are, he claims, entirely inadequate at present for the general public, and the conventional public inquiry is, too narrow in its scope to allow for truly informed decisions. In his opinion, the research council system could be of value in providing independent and authoritative data for such enquiries.

SRC appointment

The UK Science Research Council last week announced the appointment of Mr A. M. Adye (49) as its first Director of Marine Technology. He became Director on 2 May and is on second-

ment from British Petroleum Ltd.

The SRC's Marine Technology programme itself is new: it was announced only last year. Its aim is to involve academic institutions in all aspects of marine technology and to encourage postgraduate engineers to take up opportunities in the exploitation of the sea and sea-bed resources. Eventually, the Marine Technology Directorate hopes to promote the development of centres of expertise in marine technology in universities and polytechnics.

A Management Committee of six members will determine the outline of the programme, and three assessors from the Department of Energy, Department of Industry and Ministry of Defence will also be involved.

Energy body announced

The UK Secretary for Energy, Mr

Benn, announced last week the composition of the Energy Commission which is being set up to advise the government on the development of an energy strategy for Britain.

Seven members of the 23-man Commission will be the chairmen of the National Coal Board, the Electricity Council, the British Gas Corporation, the United Kingdom Atomic Energy Authority, the British National Oil Corporation, the South of Scotland Electricity Board and the Petroleum Industry Advisory Committee. Seven others will be drawn from the TUC Fuel and Power Industries Committee, and the remaining seven, whose names will be announced shortly, will represent other industries and consumer interests. The Minister of State for the Scottish Office will also be a member and the chair will be taken by the Secretary of State for Energy.

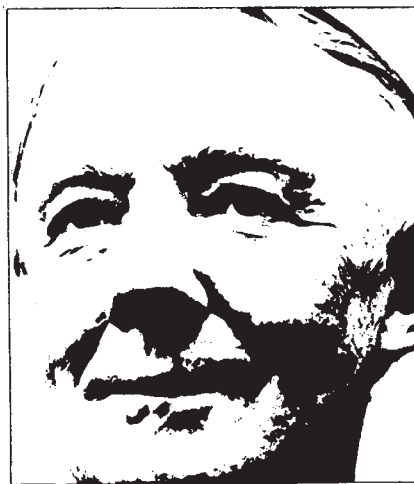
As an entomologist I spent many years killing insects. During the 1939-45 war I played some part in controlling medical pests in Britain, Africa and South East Asia, and was involved in operations where large amounts of toxic insecticides were widely and effectively distributed. I have also had some experience with agricultural pests, and nuisance problems such as swarms of gnats breeding in sewage plants. I was not the friend of the insect world, and so should not have been surprised when I suffered from its attentions.

As an undergraduate, I took part in an expedition to the Canadian Arctic. We made botanical collections and anthropological measurements of Eskimos, but it was the biting insects which impressed me most and which probably diverted my career from either medicine or botany. The most effective repellent then available was citronella oil, but liberal applications of it, a thick veil and army puttees did not prevent me from receiving thousands of mosquito bites. There are probably drops of blood from my wounds on the dried plants which were eventually sent to the herbarium at Kew in England.

Another revolting creature which has added to my sufferings is the bed-bug. As a young research student at the London School of Hygiene and Tropical Medicine, I lived in a seedy flat in Soho which we kept reasonably bug free, but on hot summer nights these insects, moving out of less well maintained property, crawled in at the windows until these were surrounded with sticky bands of the grease used to stop pests climbing up fruit trees. I met bedbugs in every

country I visited—in wicker chairs in Ceylon, where they bit the backs of my bare knees, in a rather grand hotel in Calcutta where I caught over 200 in an hour and sent them to the

The biter bit



KENNETH MELLANBY

manager with my compliments, and even in Stockholm, Sweden, in a cheap hotel in a picturesque part of the old city, now replaced by hideous modern buildings.

Then about ten years ago I lectured to the British Association for the Advancement of Science on 'Insecticides'. Halfway through my talk I felt a painful jab in my scalp; I had been stung by a wasp. With true British *savoir faire* I remarked, casually, to my audience, "I've been stung by a wasp", and continued my lecture. But somehow the press got to know, and next morning the

national newspapers contained the story with the headline: 'Insect world strikes back'.

And now it has struck again. This summer my seventeenth century farmhouse in rural England has developed a massive attack of Death Watch Beetle. I was laid up in bed for a few days, and on a warm afternoon towards the end of May I observed hundreds of these beetles emerge from what the house agents would call the 'wealth of old oak beams'. They crawled actively in all directions, and, when the temperature was above 22 °C, many flew vigorously and dashed themselves against the window panes. It was only the bedroom I occupied that produced swarms of beetles; the rest of the house yielded very few. The attack was obviously aimed at me, personally.

In some ways this is a bit unfair. For the last few years I have mended my ways, and have been mainly involved in wildlife conservation. I have helped to control the use of the more dangerous insecticides, as part of an attempt to safeguard the environment. I have banned pesticides from my garden, and have relied on the inefficient help of beneficial insects. But the Death Watch Beetle has gone too far. Now I have willingly agreed to have my house impregnated with one of the organochlorine insecticides. This is being done with care, and I hope that little will escape to harm the birds and insects in the garden. But this is war to the death, and nature must take its chance. I am even reconsidering my policy for pest control in our garden.

news and views

Population genetics and cultural inheritance

from Robert M. May

MANY of the central questions of our time, both at the barricades and in the ivory tower, turn upon the relative contribution made by genes and by environment to the behaviour of individuals.

Disentangling these factors in any specific situation is often made forbiddingly difficult by the fact that the contribution of nature depends on nurture, and that of nurture depends on nature. Medawar (*New York Review of Books* 24 (1), 13; 1977) gives an illuminating example. The little brackish water shrimp *Gammarus chevreuxi* begins life with red eyes, but usually ends up with black eyes. This blackness arises from the deposition of melanin in the eye, a process which depends both upon the genetic predisposition to form and deposit melanin, and upon the temperature and other features of the environment. Particular genotypes can be chosen such that the eye colour is determined wholly environmentally (black at relatively high temperature, red at low); conversely, it is possible to choose temperatures such that the eye colour is inherited in perfect Mendelian fashion, like the classic peas. The simple question 'what percentage of colouration of the eye is due to heredity?' is meaningless. Of course, for cases like this shrimp, or for most plant and animal breeding, the relative contributions of nature and nurture can be sorted out by careful programmes of study and experiment. For human societies, the relative roles of heredity and culture (or, to use a word newly minted by Dawkins in *The Selfish Gene*, Oxford, 1976, of genes and 'memes'), are manifestly more difficult to disentangle. Furthermore, as Medawar has lamented (*op. cit.*): "The subject is bedeviled more than any other by the tendency of disputants to spring into political postures which allow

them no freedom of movement. Thus it is a canon of high tory philosophy that a man's *breeding*—his genetic makeup—determines absolutely his abilities, his destiny, and his deserts; and it is no less characteristic of Marxism that, men being born equal, a man is what his environment and his upbringing make of him. The former belief lies at the root of racism, fascism, and all other attempts to 'make nature herself an accomplice in the crime of political inequality' (Condorcet) and the latter founders on the fallacy of human genetic equality ('A strange belief,' said J. B. S. Haldane—a long-time member of the CP)."

It would be nice if we could turn to theoretical population genetics for help. The mainstream of this subject, however, is devoted to increasingly elaborate studies of the way gene frequencies behave under the influences of mutation, migration, drift and abstract selective forces; the selection is rarely tied to concrete environmental realities.

Models for cultural inheritance

Of great interest, therefore, is the recent work of Feldman and Cavalli-Sforza (*Theor. Pop. Biol.* 4, 42, 1973; *Amer. J. hum. Genet.* 25, 618, 1973; *Annls hum. Biol.* 2, 215; 1975; see also Karlin & Carmelli *Proc. natn. Acad. Sci. U.S.A.* 71, 4727, 1974; and Lewontin *Am. J. hum. Genet.* 26, 400, 1974). Although still in the formative stages, this work treats models for cultural inheritance, and seeks to show how traits may evolve under combined genetic and phenotypic transmission.

The most recent of these studies (Feldman & Cavalli-Sforza *Theor. Pop. Biol.* 9, 238; 1976) begins with the familiar artifice of a trait that depends on one locus with two alleles, *A* and *a*, thus leading to the three genotypes *AA*, *Aa*, *aa*. Now comes the twist: adult individuals are characterised not only by their genotype, but also by whether or not they possess a certain unspecified skill. There are thus six

possible phenotypes, the skilled ones *AA*, *Aa*, *aa* and the unskilled ones *AA*, *Aa*, *aa*. For the initial study, one parent, who may or may not be skilled, is arbitrarily defined as the "teaching parent". In general, the 'educability' of the offspring will depend on its genotype so that the probability that a given individual will grow up to be skilled now depends both on the genotype of the individual (his nature) and on whether the teaching parent is skilled (his nurture). Finally, it is supposed that selection occurs; the six different phenotypes will have relative reproductive success rates that depend on their genotypes and on their acquisition of the skill.

The assumption is now made (more for convenience than from conviction) that mating occurs at random with respect to skill and genotype. Equations may then be constructed relating the frequency of the genes *A* and *a*, and the proportions of skilled compared with unskilled individuals for each of the three genotypes, between successive generations. In their most general form, these equations are highly complicated: I shall return to this below.

Feldman and Cavalli-Sforza extract interesting results by making various simplifying assumptions. In the first case they assume that the selection coefficients depend only on whether the individual is skilled or not; the relative reproductive success of any skilled to any unskilled phenotype is $1+s:1$, independent of the genotype. The genotype is of course still important in determining the probability that the phenotype will be skilled (by influencing the 'educability' of the individual), but the selection coefficients do not depend on the genotype as such. On the assumption that unskilled teaching parents never raise skilled offspring, it can be shown that the maintenance of genetic polymorphism (both *A* and *a* present at equilibrium) requires not only that heterozygotes (*Aa*) be more receptive to the skill of the parent

than are the homozygotes (AA and aa), but also that this 'overdominance' in heterozygotes' learning ability must exceed some threshold that depends on the selection parameter s . The authors give as a numerical example the case where a skilled teaching parent raises 70% of its AA or aa offspring to be skilled compared with 80% of the Aa offspring; polymorphism is maintained only if the selective margin in favour of skill exceeds $1/3$ (that is, $s > 1/3$; $1+s:1>4:3$). This is in contrast with conventional population genetics, where heterozygote superiority ensures stable polymorphism.

In the second case, Feldman and Cavalli-Sforza make a further simplification and assume that all offspring of skilled teaching parents are skilled. There is now completely accurate copying of the teaching parent (skilled or unskilled) by all offspring, irrespective of genotype. Nurture is all-important; nature is irrelevant. Obviously, the final state is for all the population to be skilled (providing the skill is advantageous, $s > 0$). Less obviously, there is no unique value for the frequency of the genes A and a ; rather, there is a neutral equilibrium, with the gene frequency depending on the initial distribution of the skill effects. Even though there are no genetically based selection differences, substantial changes in gene frequency can occur over time if there are initial differences in the distribution of the skill among the genotypes. As an example, the authors assume that the alleles A and a are initially equally common, and that the initial fractions of the genotypes AA , Aa and aa that are skilled are 90%, 50% and 10% respectively: this system will eventually settle to have the frequency of the alleles A and a around 65% and 35% respectively, corresponding to a 30% change in gene frequency. To the unsophisticated observer, it might appear that the 'superior' gene A has asserted itself; in fact, this relatively large gene frequency change is due to a kind of founder effect in a situation where all genotypes are equivalent.

Up to this point, it has been assumed that the probability of acquiring the skill (with consequent selective advantage s) depends on one 'teaching' parent. The authors next consider the case where both parents affect the outcome. To keep things manageable, the genotype of the offspring is assumed to be irrelevant; the probability that an individual will grow up to be skilled depends only on the phenotype of both parents. In these cases, the gene frequencies of A and a approach some neutral equilibrium, determined by initial values. In contrast, the phenotypic proportions of skilled and

New shocks in northeastern Alaska

from Peter J. Smith

EARTH scientists have often displayed a tendency to interpret a lack of data prematurely in terms of simplicity, presumably through an over-literal application of Occam's Razor. Only a few years ago, for example, it was not uncommon to find the upper mantle being described without qualification as physically homogeneous; and yet as more and more data have accumulated the illusion of homogeneity has receded. This happens to be one of the classic examples of oversimplification through ignorance, but there are many hundreds of others of various degrees of importance, one of which has now been revealed for what it is by Gedney *et al.* (*Geophys. Res. Lett.* 4, 175; 1977).

This new case, which concerns the seismicity of northeastern Alaska, is perhaps of minor importance scientifically but warrants attention because of its political overtones. In April 1976 Meyers (*NOAA Technical Memorandum EDS NGSDG-1*) could find mention of only 10 earthquakes having occurred in Alaska north of 68°N throughout the whole of recorded history, thereby apparently confirming the widely held view that northeastern Alaska is relatively aseismic. The fact is, however, that the first seismograph station in Alaska was not constructed until 1935, and from then until 1966 the station closest to the northeastern corner of the state lay several hundred kilometres away near Fairbanks. Moreover, even as recently as 1975 there was no station closer than Fort Yukon on the Arctic Circle. In short, the detection threshold for earthquakes in northeastern Alaska has traditionally been high.

But all that changed during 1975-1976 when Gedney and his colleagues installed nine new seismographs in the Brooks Range. This sounds easier than it was, for access to much of this area is by helicopter only. Maintenance during the winter months is therefore impossible; and the recording equipment has been usable for only about half of the time. Nevertheless, during 1976 no fewer than 69 earthquakes in the magnitude range 1-4 were recorded and located (44 of them north of 68°N) and many others were detected but could not be precisely located because of low shock magnitude or equipment failure. In other words, the result of a drastic reduction in the threshold detection limit (from about magnitude 4 to magnitude 1) has been to erase the mistaken impression that the northeastern corner of Alaska has a very low seismicity.

This may not be the first time that an area has been shown to be more seismic than previously supposed. The point about northeastern Alaska, however, is that it is the site of the northern section of the oil pipeline and of the Alaskan section of the proposed natural gas pipeline through Canada. The northern part of the oil pipeline has in fact been designed to withstand shocks in excess of magnitude 4. But discoveries such as that by Gedney *et al.* are bound to strengthen the environmentalists' insistence that any new pipeline projects should be deferred until the data available are adequate to rule out even nastier surprises.

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unskilled individuals converge to equilibrium values that depend on s and on the probabilities for given parental combinations to produce skilled offspring. Depending on the relations between these parameters, there may be a unique stable state, or two alternate stable states, or even (in rather extreme circumstances) stable cycles in the proportions of skilled phenotypes.

Disadvantageous 'cultural' traits: the 'kuru model'

In the models where acquisition of the skill depends on both parents, it is interesting to consider the case where the 'skill' confers a disadvantage ($s < 0$). Such could be the case where an infectious disease or other such disadvan-

tageous trait is transmitted from parent to offspring. Feldman and Cavalli-Sforza show that 'a strongly disadvantageous custom can spread through a group provided there is enough pressure on the members of the group to conform to the custom' (page 257). The authors refer to this as 'the kuru model', after the degenerative brain disease found among the South Fore linguistic group in the Eastern Highlands of New Guinea, and thought to derive from a slow virus (similar to that producing the rare Creutzfeldt-Jakob disease) transmitted by the custom of eating or sniffing the brain of dead relatives. The authors add that 'in these cases it would be of considerable interest to introduce the added feature of finite population size in order to

consider the problem ecologically, as one of potential extinction or survival of the group'. Indeed, kuru probably speaks more to this *caveat* than to the basic model, as the disease is now thought to have appeared around 1910 (Burnett & White *Natural History of Infectious Disease*, Cambridge, 1971; Marsh in *Slow Virus Diseases of Animals and Man*, ed. Kimberlin, North-Holland, 1976), and it is hard to see that a group could long sustain a custom with such damaging consequences; during the peak incidence of the disease, in the 1950s, 1% of the Fore population would die of kuru each year, with the prevalence of active kuru reaching 5–10% of the inhabitants of certain tribes (Marsh *op. cit.*).

The authors also show that if any strongly disadvantageous trait can be learned outside the family environment, then a low frequency of the trait may be maintained, in a manner completely analogous to the classical mutation–selection balance of population genetics.

Formidable mathematical difficulties

stand in the way of a more full understanding of the interplay between cultural and biological evolutionary processes. As noted by the above authors, and, in a related context, by Oster, Ipaktchi and Rocklin (*Theor. Pop. Biol.* **10**, 365; 1976), by Slatkin (*Proc. natn. Acad. Sci. U.S.A.* **66**, 87; 1970) and by Lande (*Genet. Res. Camb.* **26**, 221; 1975), the general equations relating the gene frequencies in successive generations are not only non-linear, but also involve frequencies from yet earlier generations. The resulting dynamics can have alternate stable points, or a variety of stable cycles, or even apparently chaotic fluctuations that are in many ways indistinguishable from random noise (see the reviews by May *Nature* **261**, 459; 1976; and by May & Oster, *Am. Nat.* **110**, 573; 1976). Nevertheless I believe that the incorporation of cultural inheritance into the quantitative theory of population genetics, as begun by Feldman, Cavalli-Sforza and others, is likely to open exciting new areas. □

Ancient distribution of lime trees in Britain

from Peter D. Moore

Two species of lime tree, *Tilia cordata* and *T. platyphyllos*, together with their hybrid, are native to the British Isles. They are warmth-demanding trees and reach their climatic limits in Britain, neither species occurring naturally in areas with a July mean temperature less than 15 °C. Pigott (*J. Ecol.* **57**, 491; 1969) considers that woodlands containing these species have often been undisturbed over long periods of time, for they frequently contain a characteristic assemblage of plant species which is lost in disturbed woodlands. Interference by man in such woodlands, he claims, often leads to the replacement of lime by ash, elm and sycamore. Such a view was given support by the palynological work of Turner (*New Phytol.* **61**, 328; 1962), who demonstrated the coincidence of the decline in *Tilia* pollen, often in Iron Age times, with evidence of human activity.

Within the British Isles, *Tilia platyphyllos* is the more restricted in its distribution, and both species are generally associated with base rich soils of mull humus type. *T. cordata*, however, does occur on acid, well-drained soils in eastern Europe in association with pine and spruce

(Pigott *Phil. Trans. R. Soc.* **270**, 151; 1975).

The past history of the genus in Britain is rather confused for a number of reasons. Identification to specific level has only been undertaken as a routine process in recent years, since Andrew (*New Phytol.* **70**, 683; 1971) showed that exine patterning provides a reliable guide. Subsequent counts in which separation has been effected, such as those at Holme Fen by Godwin and Vishnu-Mittre (*Phil. Trans. R. Soc. Lond.* **270**, 561; 1975) show a strong predominance of *T. cordata* throughout the post-glacial, which corresponds with present-day relative abundance of the two species. Most other published and unpublished data from other parts of Britain confirm this.

Before human interference with woodland became a major factor in the history of our vegetation, say 5,000 years ago, the pollen of lime was distinctly more abundant in southern and eastern England. Birks, Deacon and Peglar (*Proc. R. Soc. Lond. B* **189**, 87; 1975) have mapped *Tilia* pollen frequencies at that time and show only East Anglia, the east Midlands and the south-eastern extremity of Britain with values of 11–15% of tree pollen. There were times, however, during the

height of forest development in this country when lime must have been locally abundant. In East Anglia at Holme Fen, values up to 50% arboreal pollen were recorded, and up to 30% at Old Buckenham Mere (Godwin *New Phytol.* **67**, 95; 1968).

Being insect pollinated, *Tilia* is probably under-represented in aerial pollen fallout, although according to Godwin (*History of the British Flora*, Cambridge University Press; 1975) it produces large quantities of pollen. It is difficult, therefore, to interpret such values in terms of lime abundance in the contemporary woodlands.

It is interesting to note that high *Tilia* proportions have also been recently reported from areas on Cretaceous and Tertiary sands and gravels in the south east. For example, unpublished analyses of J. A. Webb on Hothfield Common, Kent (Lower Greensand) have *Tilia* cf. *cordata* pollen values up to 40% of arboreal pollen. On the northern side of the North Downs from this site, at Wingham, near Canterbury, a value of 20% was obtained by Godwin (*Veröff. geobot. Inst. Zürich* **37**, 83; 1962). In this analyses of Wealden soils, however, Dimbleby has failed to record high *Tilia* pollen levels (see for example, *Proc. Prehist. Soc.* **26**, 246; 1960; **31**, 85; 1965; *Grana Palynol.* **4**, 140; 1963, all sites on the Wealden Lower Greensand). It is interesting to note in passing that one of Dimbleby's sites, Addington in Kent, was the location of the first reliable record for sub-fossil *Tilia platyphyllos* by Burchell and Erdtman (*Nature* **165**, 411; 1950).

On the North side of London, some unpublished analyses of the small valley mires of Epping Forest by P. Oxford have revealed high *Tilia* levels in basal samples. Now, in this issue of *Nature* (page 45), Girling and Grieg have published a pollen diagram from the Bagshot Sands of Hampstead Heath, and they also find high levels of lime pollen (up to 30% of arboreal pollen excluding *Alnus*). The exclusion of *Alnus* from the pollen sum has little effect upon these high *Tilia* levels below 100 cm in depth as *Alnus* abundance is low below this. On the basis of this and other data Girling and Grieg suggest that lime was a dominant tree in southern and eastern England. Locally this may well be the case, but the great variation found from one site to another does indicate that high *Tilia* representation is likely to reflect only local dominance. The isolated records of abundant lime pollen from sites further north and west, such as

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Shustoke, Warwickshire (Kelly & Osborne *Proc. Linn. Soc. Lond.* **176**, 37; 1965) and west Wales (Smith & Taylor *Trans. Inst. Br. Geogr.* **48**, 75; 1969) show that there may have been local pockets of lime dominance outside the south east of England. But until more data is available from

southerly sites the extent of lime dominance will remain an area of speculation. Results such as those of Girling and Greig make it evident that many sandy areas in the south east which now bear heathland were once covered by forest at least locally rich in lime. □

Amyloidosis, P component and complement

from A. H. Gordon

AMYLOIDOSIS is the pathological deposition of glycoprotein-like material in body tissues. Various genetically different types of the disease have been identified, in which the major amyloid component and the sites of deposition differ. In primary amyloidosis (in which there is no obvious predisposing condition) immunoglobulins are apparently the main constituent of amyloid fibrils. In secondary amyloidosis, which is often a complication of chronic destructive infections such as tuberculosis and leprosy, the fibrillar AA protein deposits are immunologically similar to one particular plasma protein, SAA, (also found in normal sera) synthesised by B cells. Recent work on the analysis of amyloid tissue deposits has also revealed close amino acid sequence similarities between P component, present as a minor component in all forms of the disease and the recently discovered C1t, the fourth constituent of the first component of complement (C1). A more distant but definite sequence homology has also been demonstrated between P component and CRP (C-reative protein) (Levo *et al.* this issue of *Nature* page 56), a plasma protein which can interact with pneumococcal C-polysaccharide, choline phosphate and various polycations to activate the complement cascade. Although a great deal is now known about amyloid components, the factors which cause amyloid deposition are still unclear.

In secondary amyloidosis for example, the concentrations of many plasma proteins, especially the so-called 'acute phase' proteins are increased, SAA among them. Acute phase proteins are those which are found in the serum in increased concentrations after any injury or shock. Amyloidosis can be induced in susceptible strains of mice by repeated injections of casein or lipopolysaccharide but McAdam and Sipe have shown that although injection of a single dose of bacterial lipopolysaccharide or casein (B lymphocyte mitogens) in mice leads to greatly increased concentrations of

SAA in the plasma, this does not always lead to amyloidosis (*J. exp. Med.* **144**, 1121; 1976). They consider that in this case the difference between amyloid-prone and resistant mice is likely to be found in the lysosomes. According to this view the amyloid-prone mice are considered to suffer from a catabolic defect in protein degradation.

An interesting difference between SAA obtained from amyloid and non-amyloid human plasma has also been reported by Sipe *et al.* (*Br. J. exp. Path.* **57**, 582; 1976). This concerns the β -pleated sheet conformation which renders the protein of the fibrils insoluble. The difference relates to the dissociation in guanidine HCl or formic acid of SAA of molecular weight 160,000 to SAAL (molecular weight 12,500). In the same conditions SAA from amyloid plasma dissociated less, and reaggregated more readily, than SAA from normal plasma.

Because the increased concentration of SAA which occurs in these conditions will be accompanied by increased concentrations of a number of other plasma proteins, it is necessary to consider how far increased concentrations of other acute phase plasma proteins may predispose towards amyloidosis. In rabbits and humans the acute phase protein which increases most is CRP. As mentioned above, this protein shows partial amino acid homology with the P component of amyloid which is itself homologous with C1t. CRP, P component and C1t all complex strongly with Ca^{2+} . Furthermore, in inflammatory conditions the opportunity exists for CRP to complex with a number of cationic and/or anionic products of a cell breakdown or, indeed, with C-polysaccharide itself.

Such complexes act very similarly to antigen-antibody complexes in that they bind to C1 and initiate the consumption of complement. Perhaps the most surprising fact to emerge from the structural studies is the extensive homology between CRP, which itself is an activator of C1, and C1t, one of the

subcomponents of C1. Since both CRP and C1t react with C1q they may in some manner modulate each others actions. This may affect the availability of P component and thus the likelihood of amyloid deposition.

These structural relationships, if seen from the evolutionary standpoint, take on a wider immunological significance. Also important in this regard is a further homology recently shown to exist by Osmand, Gewurz and Friedenson (*Proc. natn. Acad. Sci. U.S.A.* **74**, 1214; 1977) between CRP and the immunoglobulins. Comparison of the sequences of CRP and the $\text{C}_{\text{H}}3$ domain of human IgG has indicated a distant but significant homology. Using in addition data for C1t, the times since divergence of CRP and C1t have been calculated to be 2×10^8 yr, and more than 5×10^8 yr, for the earlier divergence of CRP from the immunoglobulins. The structural relationship now apparent between CRP and the immunoglobulins might perhaps have been expected in view of the similarity of function of antibody-antigen complexes and CRP when complexed with C-polysaccharide or another polyanion or polycation. These include the ability to stimulate phagocytosis and act both as agglutinin and opsonin. Particularly relevant to amyloidosis, however, is the activation of complement by both antigen-antibody complexes and CRP in complex form. Certainly long before divergence of CRP and C1t took place the parent molecule which may be presumed to have already had the power to bind Ca^{2+} would have been available as a constituent of amyloid.

Further evidence for the formation of amyloid from α_2 globulins of mouse plasma has been obtained by Kisilevsky *et al.* (*Am. J. Path.* **83**, 299; 1976). In this work with ^{125}I -labelled mouse plasma proteins, rates of synthesis and degradation of albumin α_1 , α_2 , β and γ globulins were measured for groups of mice receiving azocasein (AC) and amyloid-enhancing factor (AEF) obtained from the spleens of mice previously treated with AC. Amyloid was found only in the group which had received AC and AEF. However, both this group and that which had received AC only, showed significant increases in the turnover rate of α_2 globulin, $t^{1/2}$ 73% and 79% respectively, compared with the control group. This increased turnover of α_2 globulin by both groups can be reconciled with the appearance of amyloid in the AC+AEF group only, on the assumption that amyloid deposition occurs by removal of partially degraded material and that this process is without effect

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on the turnover rate. On the basis of these experiments Kisilevsky has attempted a generalisation as to the mechanism underlying amyloid deposition. In his view the precursors of amyloid are *I*-chains in myeloma-associated amyloid and α_2 -globulin when the disease is secondary to an inflammatory state.

In such conditions an increased load of glycoproteins, which will be accounted for largely by the increased concentrations of SAA, will be taken up by the reticuloendothelial system (RES). If the RES is overloaded or functioning less than optimally, as for instance after treatment with BCG, degradation of α_2 globulins, and in particular SAA, will not go to completion. Thus, subunits will then accumulate and in due course will combine to form fibrils. However, for this to happen certain additional conditions are likely to be necessary. Since the partial breakdown of the SAA protein or other glycoproteins will occur in the lysosomes differences in respect to lysosomal enzymes are likely to be important. The existence of genetic differences in respect to amyloidosis has already been mentioned. Minor genetically-determined structural differences may perhaps be responsible for the enhanced tendency to associate as amyloid fibrils shown by the SAA obtained from amyloid plasma. □

Anoxic events in the Cretaceous ocean

from A. Hallam

THAT the Black Sea is stagnant in its deeper part is a widely known and comparatively well understood fact. Freshwater runoff from the surrounding land promotes salinity and hence density layering which is not destroyed by convective overturn because of restriction on interchange with Mediterranean waters by the sill of the Bosphorus. The surface waters contain free oxygen because of photosynthetic activity and exchange with the atmosphere, but at greater depth an anaerobic condition is produced as oxygen is consumed by organisms and replaced by hydrogen sulphide, the by-product of the metabolic activity of sulphate-reducing bacteria. In consequence the bottom sediments are rich in unoxidised bituminous matter derived both from terrestrial plants and phytoplankton, together with iron sul-

Terminal redundancy in avian oncornavirus RNA

from T. J. R. Harris

THE avian oncornaviruses provide a good model for analysing the *modus operandi* of the Retroviridae. Viruses in this family make a DNA copy of their single stranded genome RNA using the RNA-dependent DNA polymerase (reverse transcriptase) packaged in the virus particle. This complementary DNA (cDNA) is then copied into a circular double-stranded DNA molecule which can integrate into the host cell DNA. Studies *in vitro* with reverse transcriptase are now beginning to unravel the possible molecular mechanisms behind this interesting sequence of events and to explain how the circular DNA molecules might be formed.

The primer required by reverse transcriptase for the initiation of synthesis is one of the host cell transfer RNA molecules associated with the genome RNA and making up the 70S complex in the virus. For both Rous sarcoma virus (RSV) and avian myeloblastosis virus (AMV), the tRNA specific for tryptophan is the one used. As cDNA synthesis proceeds in a 5'–3' direction one might expect that the primer would be bound at the 3' end of the RNA. Surprisingly, however, Taylor & Illmensee in a concise paper (*J. Virol.* **16**, 553; 1975) showed for RSV RNA that the primer was in fact bound near the 5' end of the RNA—a finding since confirmed for AMV RNA (Stakus *et al. Virology* **71**, 162; 1976). Collett & Faras (*Proc. natn. Acad. Sci. U.S.A.* **73**, 1329; 1976) analysed the cDNA produced by the AMV enzyme and found that molecules containing sequences complementary to the 5' end of the virus RNA also contained sequences complementary to the 3' end. These observations led the authors to suggest that some of the sequence at the 5' end of the virus RNA was repeated at the 3' end providing both virus RNA and cDNA with the capacity to form circles. Direct evidence has now been obtained from sequencing studies to support this suggestion. Haseltine, Gilbert & Maxam (*Proc. natn. Acad. Sci. U.S.A.* **74**, 989; 1977) have

sequenced the 101 nucleotides of tRNA^{Trp}-primed cDNA complementary to the 5' end of the virus RNA using their rapid DNA sequencing technique, while Schwartz, Zamecnik & Weith (*Proc. natn. Acad. Sci. U.S.A.* **74**, 989; 1977) used oligo(dT)-primed reverse transcription followed by partial exonuclease digestion of the product to obtain a short sequence adjacent to the poly(A) at the 3' end of the virus RNA. The first 21 nucleotides of RSV RNA after the capping group were found to be identical to the 21 before the poly(A). Haseltine *et al.* discuss in detail the implications of the terminal redundancy for circle formation and formation of provirus DNA and some evidence for the circular structures they envisage is accumulating from heteroduplex analysis of RNA/cDNA hybrids (see Junghans *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 477; 1977). Also, knowledge of the 5' cDNA sequence enabled Haseltine *et al.* to construct plausible models of the possible ways that the 5' ends of the two genome RNA molecules and their tRNA primers might be associated in the virus particle as the 70S complex.

The advent of the fast and accurate nucleic acid sequencing methods should enable more of the cDNA sequence to be determined (as well as that of the dsDNA and its precursor molecules) so that some of these speculations can be tested. One must add the proviso however that any mechanism of synthesis outlined from *in vitro* studies may not necessarily be the same as that actually occurring *in vivo*. Nevertheless, sequencing the parts of the RNA (by way of cDNA) coding for the structural and non-structural polypeptides will inevitably lead to a greater understanding of the replication of this important family of oncogenic viruses.

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phide. They are also finely laminated because no benthonic organisms can survive in the anoxic environment and the sediment is not churned over by burrowers. Because of the name by which the Black Sea was known in the classical world, such sediments and environments are known as euxinic,

and a Black Sea-type 'barred basin' model has been widely applied by geologists to account for the formation of finely laminated marine bituminous shales.

In marked contrast to the rather unusual Black Sea situation, the floor of the open ocean is well oxygenated,

with powerful currents and water renewal operating even at great depth. It has therefore come as one of the great surprises of the Deep Sea Drilling Project that, at several horizons within the Cretaceous, bituminous sediments comparable with those of the Black Sea have been discovered beneath the Atlantic, Indian and Pacific oceans, with an organic carbon content as high as 28%. In the case of the earliest discoveries in the Atlantic, research workers initially proposed a Black Sea model, with stagnant basins in a much smaller ocean possessing only restricted connections with the larger ocean system. Now that it is known that such organic-rich deposits are much more widespread, and occur also on topographic rises in the Pacific, such an interpretation must be abandoned.

A number of recent papers is devoted partly or entirely to the problems posed by these deposits (Ryan & Cita *Mar. Geol.* **23**, 197; 1977: Schlanger & Jenkyns *Geol. Mijnb.* **55**, 179; 1976: Fischer & Arthur, *Soc. Econ. Min Paleont. Spec. Publ.* **25**, in the press). On one major point all the authors are in agreement. The Cretaceous world ocean must have been in a very different state from that of today, with a considerably expanded oxygen minimum zone. The oxygen content in the modern ocean diminishes rapidly downwards in quite shallow water, but increases again in the depths. This pattern relates to strong circulation of cold and dense bottom waters from surface water at the poles and is therefore ultimately bound up with climate. In more equable periods in the past such as the Cretaceous, with no polar ice caps, oceanic circulation would have been much more sluggish, with a far lower rate of renewal of bottom waters.

Beyond this, the authors diverge in their treatment, though not necessarily in a mutually contradictory way. Ryan and Cita cite four principal horizons where extensive organic-rich sapropels have accumulated, in the latest Barremian, Aptian, mid-Cenomanian and latest Coniacian stages, and calculate that they contain an order of magnitude more carbon than is present in all the known reserves of coal and petroleum. They therefore form an enormous sink for carbon and also sulphur (because of the abstraction of iron sulphide). Ryan and Cita argue for significant increase, in consequence, in the amount of atmospheric oxygen. Schlanger and Jenkyns single out Aptian-Albian and Cenomanian-Turonian boundary beds as being especially important, and point to similar bituminous deposits occurring at these horizons also in quite shallow water deposits on the continents. They attribute these unusual

and evidently extremely widespread occurrences to eustatic rises of sea level in a more equable world with a wider tropical belt than at present, with in consequence much higher organic productivity both on land and in the sea, and infer a pronounced oxygen minimum layer which reaches up to less than 300 m and down to 2,000–3,000 m below sea level.

For Fischer and Arthur, the Cretaceous bituminous deposits are an important component of an ambitious and imaginative hypothesis, which distinguishes a series of polytaxic and oligotaxic episodes extending back to the Jurassic with a 32 Myr cycle. Their polytaxic episodes are characterised by high organic diversity, higher and more uniform oceanic temperatures, eustatic rise of sea level, widespread marine anaerobism and undisturbed marine sedimentation, together with heavier carbon isotopes in organic matter. Oligotaxic episodes including the present day, exhibit, in contrast, lowered organic diversity, low sea level, sharper lateral and vertical temperature gradients in the oceans because

of stronger circulation, more disturbed and aerobic sedimentation and lighter carbon isotopes. The fundamental controls are seen as climate and relative height of sea level.

To establish a 32 Myr cycle Fischer and Arthur seem to be guilty in some cases of pushing the evidence beyond reasonable limits. For example, they are obliged to stress the importance of the Albian bituminous horizon at the expense of the others, and choose to ignore the fact that in north-east England the stage is developed in red chalk facies, one of their markers for oligotaxic episodes. Furthermore, the Tertiary bituminous occurrences they cite are much less well established in the oceans than those of the Cretaceous. While there are indeed some striking correlations between diversity, sea level and isotope abundances in the Tertiary, the evidence presented for the Jurassic looks decidedly unconvincing. Nevertheless these reservations should not be held seriously to detract from a highly stimulating and thought-provoking work, which ought to be read by those interested in ocean history. □

New interest in carbon lamellar compounds

from A. R. Ubbelohde

A Conference on Carbon Lamellar Compounds was held at Napoule, near Cannes, on 23–27 May 1977. It was jointly organised by Professor A. Herold, Department of Applied Mineral Chemistry of the University of Nancy, and Professor F. Vogel, Moore School of Electrical Engineering of the University of Pennsylvania at Philadelphia. The Conference Proceedings will be published as a special issue of the *Journal of Materials Science and Engineering*, which will be obtainable separately. This article aims to stress some of the more striking information presented and discussed.

WAVES of innovative international research on graphites have passed through at least two previous maxima. The first wave was powered by interest in uses of graphite in nuclear energy generation. Gradually this has tapered to an asymptote, with the realisation

that such uses have foreseeable limitations. A second wave was associated with the brilliant promise of carbon fibres for the aircraft industry. Following a spectacular setback, slow recovery of research and development on carbon fibres is under way. Though at present rather humdrum, and prompted by applications of carbon fibres to golf clubs and fishing rods, such background research seems likely to grow steadily.

A third wave of very active innovative research on graphites has formed internationally in recent years. It concerns the remarkable properties of their lamellar compounds. These are formed by intercalation of a great variety of molecules between the carbon hexagon layers in graphite, after these have been separated sufficiently for the intercalate species to enter and lie between them. Although the conference reported here dealt predominantly with graphite compounds, other layer crystals such as MoS₂ show analogous behaviour. From a chemical standpoint, intercalates can broadly be grouped as electron donors, such as the alkali metals Li, (Na) K, Rb and Cs, and electron acceptors such as Br₂, ICl, inorganic halides, and a number of other types. There is also an important group of carbon lamellar

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compounds such as graphite bisulphate $C_{24}H_{50}SO_4 \cdot 2H_2SO_4$, formed by electrochemical (or the equivalent chemical) oxidation in the presence of the appropriate acid molecules.

Synthetic metals

One basic reason for the current widespread interest in lamellar compounds stems from the fact that in these the partner molecules form charge transfer bonds with the carbon hexagon networks, which act as giant aromatic molecules in this respect. Such charge transfer bonding was first postulated some 25 years ago (McDonnell *et al.* *J. chem. Soc.* 191; 1951). Many examples have now been studied. The striking growth of research on these compounds seems to be due to their interest and promise as synthetic metallic conductors. Intercalate molecules in carbon lamellar compounds also show distinctive chemical behaviour as catalysts, and in other ways. Means of making various precise physical and optical measurements have become more refined in recent years, so that much more precise information is beginning to be available to help interpret the nature and properties of the charge transfer bands formed. Nearly one-third of some 60 papers discussed during the meeting dealt with this aspect. Practically all the advances described were very recent. Surprising new facts and novel theoretical arguments kept members of the conference in a ferment throughout.

Inorganic chemistry

Experimental methods were described for introducing intercalate species not hitherto known to form lamellar compounds, for example by the use of oxidising agents such as $C_6F_5AsF_6$ (N. Bartlett, Lawrence Berkeley Laboratory, University of California, Berkeley) or catalysts such as $Co(AlCl_4)_2$ (E. Stumpp, Technical University at Clausthal-Zellerfeld). Production of C_6Li by interdiffusion of the powdered solid elements (Herold) is also a recent innovation in the use of Li as an electron donor intercalate. Formation of ternary lamellar compounds by intercalation of a second partner into binary lamellar compounds often occurs rather unexpectedly. Different instances described by several authors included the intercalation of molecular H_2 , of tetrahydrofurane, of benzene, or of other molecules into lamellar compounds with alkali metals. Apart from any effects on the local charge transfer bonds, such ternary intercalation usually pushes apart even further the carbon hexagon layers from their initial separation of 3.35 Å in graphite. This 'layer dissociation' may have important consequences for electrical and other

properties of carbon lamellar compounds.

Solid-state physics

Clarification of physical properties of various lamellar compounds of graphite was discussed. There have been elegant advances in band theory and important information has been obtained by precision measurements of various optical properties. For example, it now seems clear from infrared and Raman spectra that the fractional charge transfer when molecular Br_2 is intercalated is only a few tenths of one percent (G. Dresselhaus and M. S. Dresselhaus, Massachusetts Institute of Technology). Graphite/bromine was one of the first lamellar compounds for which a remarkable increase of metallic properties occurs on intercalation (McDonnell *et al. op. cit.*) and the rise in electrical conductivity begins steeply as the first few molecules of bromine enter the graphite. This recent optical work suggests (A. R. Ubbelohde, Imperial College, London) that metallic conduction in the halogen lamellar compounds resulting from charge transfer involves only relatively few additional charge carriers, but if so these must have exceptionally large mobilities.

Quite generally, under suitable conditions, synthetic metals formed by charge transfer in lamellar compounds of graphite seem to exhibit exceptionally low scattering of charge carriers travelling in the directions of the layer planes. This points to a two-dimensional mode of conduction which differs at least quantitatively and perhaps also qualitatively from that in the familiar three-dimensional metals (Ubbelohde). Such findings stress the importance of

making many more measurements on the anisotropy of various physical properties parallel and perpendicular to the layer planes. Remarkably, very few *c*-axis measurements on lamellar compounds were presented to this conference, but there seems every hope that this gap will before long be filled.

Ramsey fringes in optical spectroscopy

from Robin M. Hochstrasser

EXPERIMENTS have been reported recently in which the spectral resolution in optical spectroscopy of gases can be limited only by the time of flight difference between two light pulses. Limitations due to Doppler broadening, the homogeneous width of the resonant state, or the transform spectral width of the laser pulse, have been eliminated.

The Doppler broadening of a spectral line at ω_{ba} can be eliminated if the transition $a \rightarrow b$ is brought about by the simultaneous absorption of two photons, one from each of two counter-propagating beams having frequency $\omega_{ba}/2$. This type of non-linear spectroscopy requires high light power so that present day laser beams must be sharply focused in order for the signals to become readily detectable. With high peak power pulsed lasers such as a nitrogen-pumped dye laser some focusing of the beam is still necessary to observe these non-linear effects.

A severe limitation of pulsed dye lasers in high resolution spectroscopy is that typical pulses have a duration of 8 ns corresponding to a minimum frequency uncertainty of ~ 125 MHz for transitions occurring during the transit of such a pulse. For molecules travelling at 10^3 m s⁻¹ across a laser beam focused to a diameter of 10^{-4} m the accuracy of the experiment is not limited by the transverse transit time which contributes only ~ 10 MHz to the spectral width. Transit time limitations on spectral accuracy have been known for a long time in beam resonance spectroscopy and in 1949 N. F. Ramsey (*Molecular Beams*, Ch V, Oxford University Press, 1956) suggested his method of separated oscillating fields in order to overcome this difficulty. In Ramsey's method the molecular beam passes through an RF field region of length l in time τ , and then travels a distance L before reaching a second identical RF field region. The number of transitions at a fixed RF frequency undergone in such a spectrometer is an oscillatory function of the time L/v , where v is the most probable velocity of molecules



A hundred years ago

THE application of new materials for paper stock which has occupied so much attention of late seems to have attracted some notice in the Philadelphia Exhibition last year. From Jamaica bamboo was perhaps the most important paper-making plant exhibited. Of the young bamboo stems, which are the best for the purpose, a very large supply, it is said, could be annually, by systematic cuttings or croppings, furnished from plants flourishing in the humid parts of the island.

from *Nature* 16, 5 July, 1877.

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in the beam. In the intermediate region the separate components of the non-stationary state produced by the first RF region each evolve like stationary states for a time L/v so that the wavefunction is changed from

$$\psi(\tau) = \{a(\tau)\phi_a + b(\tau)\phi_b\}$$

into

$$\psi(\tau + L/v) = \{a(\tau)e^{-i\omega_a L/v}\phi_a + b(\tau)e^{-i\omega_b L/v}\phi_b\}.$$

The oscillations, or Ramsey fringes as they are now termed, arise because the probability of transitions in the second RF region is proportional to:

$$|A(\tau) \sin \{(\omega_{ba} - \omega)L/2v\} + B(\tau) \cos \{(\omega_{ba} - \omega)L/2v\}|^2,$$

which corresponds to an interference pattern. The fringes are separated by $V/2L$ whereas the spectra from only one RF region have widths of $\sim 1/\tau$, so that the method of separated fields improves the sharpness of the spectral lines by a factor of $\sim L/l$.

In the paper by Salour and Cohen-Tannoudji (*Phys. Rev. Lett.* **38**, 757; 1977) an analogue of the Ramsey fringes was observed in the optical region. Whereas with the RF field it is easy to have two regions excited coherently during the whole experiment, it is rather difficult to accomplish this with optical fields. Instead, Salour and Cohen-Tannoudji generated two pulses in an optical delay line starting with a pulse obtained by amplifying a cw wave in a series of synchronously pulsed dye laser amplifiers. The two phase-locked pulses, separated by a distance L , were focused into a cell containing sodium vapour behind which was a mirror to reflect the beams back through the sample. The vapour was thereby subjected to a standing wave optical field of two pulses each of width τ separated by $T = L/c$. There is no Doppler shift for two-photon transitions in the standing wavefield. A photomultiplier detected the 90° fluorescence that occurs after two-photon absorption by Na atoms. The two-photon excitation spectrum of the $3^2S \rightarrow 4^2D$ transition, obtained by scanning the laser and detecting the fluorescence, showed not only the hyperfine structure, but each hyperfine component displayed Ramsey type fringes separated by as little as 30 MHz for $2T = 33$ ns.

Teets *et al.* (*Phys. Rev. Lett.* **38**, 760; 1977) also investigated the Doppler-free two-photon spectrum of sodium using a train of phase coherent pulses generated by multiple reflections of a dye laser pulse in a resonator containing the sample. The two photon signal was measured as a function of resonator

tuning and a Fabry-Perot type spectrum was obtained with line-widths of about 12 MHz using a 2-m confocal resonator.

Although these experiments are expensive and not uncomplicated, especially in regard to the problem of maintaining a constant phase difference between the laser pulses, their success opens up important new possibilities for the study of molecular excited states at MHz resolution. $\square$

Heavy ion transfer reactions

from P. E. Hodgson

THERE have been many studies of reactions between heavy ions in which one or more nucleons are transferred, and it is usually found that the cross section can be accounted for quite well by the distorted wave theory. In some cases it is found that the cross sections are more sensitive to particular aspects of nuclear structure than the corresponding reactions with light projectiles, and this makes them useful tools in nuclear spectroscopy.

An example of this is the spin-dependent effects in some transfer reactions. If a particle is transferred from a (l_2, j_2) state in the projectile to a (l_1, j_1) state in the residual nucleus the

angular momentum selection rules allow several values of the transferred angular momentum L , and these depend on whether the angular momentum given to the residual nucleus is $j_1 = l_1 + \frac{1}{2}$ or $j_1 = l_1 - \frac{1}{2}$. In the particular case when $l_2 = 0$, then $L = l_1$ and it is possible to study the dependence of the cross section on j_1 for a given value of L .

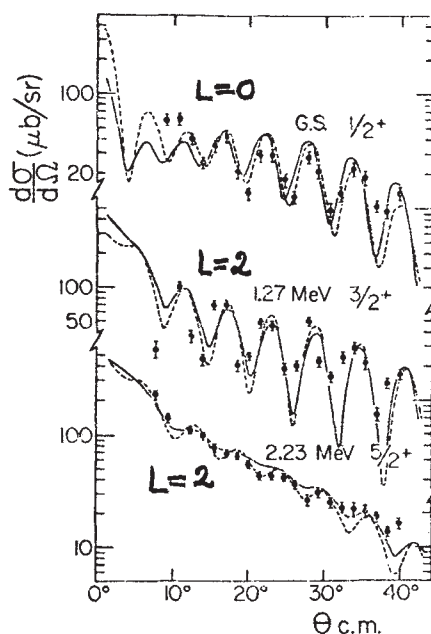
Such a study has recently been carried out by a group at the University of Minnesota for the $^{28}\text{Si}(^{19}\text{F}, ^{16}\text{O})^{31}\text{P}$ reaction at 60 MeV (*Phys. Rev. Lett.* **38**, 817; 1977). In this reaction three nucleons are transferred, but it is a good approximation to consider these as a triton-like cluster and thus retain the simplicity of a one-nucleon transfer reaction. This is likely to be quite a good approximation since 92% of the ^{19}F wavefunction can be expressed as a cluster of three particles in a relative $1s$ state moving with angular momentum $l_2 = 0$ with respect to the ^{16}O core. The experimental cross sections for the $L=2$ transfers to the lowest $\frac{3}{2}^+$ and $\frac{5}{2}^+$ states in ^{31}P are shown in the figure; these differ markedly and show how the reaction can depend on j as well as on L . The cross section for the $L=0$ reaction to the $\frac{1}{2}^+$ ground state is also shown.

The first distorted wave calculations, made with a finite range interaction and taking recoil into account, did not give a satisfactory fit to the data, and there was no appreciable improvement when inelastic excitation of the projectile was included by making a full coupled-channels calculation. The satisfactory fit shown in the figure was obtained when spin-orbit potentials were included in the distorting potentials in the incident and exit channels. Once again the fit was very little affected by the inclusion of inelastic excitations.

This work shows that the pronounced j -dependent effects in three-nucleon transfer reactions can be accounted for using the distorted wave theory provided spin-orbit potentials are included in the distorting potentials. It also provides striking confirmation of the validity of the cluster transfer approximation and shows that in this case inelastic excitations are relatively unimportant.

Further studies of similar reactions will be needed to confirm that the essential physics of the reaction has indeed been well understood, and if this is the case then the reaction, and others like it, should prove to be a powerful spectroscopic tool for determining the spins of the final states in the residual nuclei. $\square$

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Differential cross section for the reaction $^{28}\text{Si}(^{19}\text{F}, ^{16}\text{O})^{31}\text{P}$ at 60 MeV to three states in ^{31}P compared with normalised distorted wave calculations including a spin-orbit potential in the incident and exit channels. The dashed curves show the effect of including inelastic excitations.

review article

Energy production by microbial photosynthesis

John R. Benemann*, Joseph C. Weissman, Ben L. Koopman & William J. Oswald

The large amounts of microalgae produced in waste treatment ponds during sewage purification are a potential source of methane and fertiliser. If techniques can be developed for the selective cultivation of filamentous or colonial microalgae, harvesting of microalgal biomass could be accomplished by low-cost straining or sedimentation methods.

INCREASING public and governmental awareness of the serious problems of energy and environment faced by most of the nations of the world is resulting in reappraisals of all potential energy sources. Evaluations of the various energy alternatives must consider, together with technical and economic factors, their environmental and social impact. Here we review the scientific and practical aspects of fuel and fertiliser production from microalgae and photosynthetic bacteria.

Bioconversion

Until less than a century ago, photosynthesis provided over 90% of mankind's fuels¹. Even today, on a worldwide basis, the total calorific value of primary photosynthetic production (plant biomass) used by mankind is higher than that of the fossil energy consumed². Only in the industrialised nations with temperate climates are fossil fuels more important than current photosynthetic biomass production in such a comparison. At present, over one-half of the people in the world depend on wood and dung for the majority of their fuels. The diminishing availability of these biofuels to the poorer half of the Earth's population is a more serious and immediate energy crisis than depletion or monopolistic control of fossil fuels.

Photosynthesis can provide a larger share of mankind's fuel needs through (1) shifts in relative usage (such as conversion of organic wastes to fuels or reduction in luxury consumption levels of animal protein) or (2) by increasing plant biomass production for direct utilisation or conversion to fuels. The potential of plant biomass production for the exclusive or primary purpose of fuel production is, in practice, limited by a number of constraints—the relatively low efficiency of photosynthesis in solar energy conversion; competition with agriculture or forestry for land, water and fertiliser; marginal economics of biomass production and conversion into usable energy; need for low-energy cultivation technology; and a lack of experience with such processes, and so on. Because most inputs in a bioconversion system would be constant per unit area, the net productivity (expressed as incident solar energy conversion efficiency) is a critical parameter since it must be sufficiently high to justify all costs.

Photosynthesis is limited by its dependence on visible light

(about 45% of total solar radiation), reflectance losses (10–20%), a quantum efficiency of only 25%, and metabolic losses, giving a value of 5–6% for the theoretical maximum solar energy conversion efficiency^{3–7}. In normal field conditions, photosynthesis rarely approaches the theoretical limit which applies only in light-limiting conditions. Sustained year-round, solar conversion efficiencies exceeding 1% are unusual in agriculture. In addition, such high yields are often dependent on high fossil fuel subsidies or large human labour inputs, thus reducing the net efficiencies realised. Very high productivities (above 1% efficiency) with favourable net energy ratios are possible only with a few highly productive plants such as sugar cane⁸. The limitations to bioconversion suggest that practical applications will require utilisation either of the most efficient agricultural crops (for example sugar cane) or less efficient but more hardy plants capable of being cultivated in marginal areas with few inputs. The list of plants considered for biomass production is already large; they may be classified as either dry land or aquatic. The aquatic plants may, in turn, be classified as macro (water hyacinths, seaweeds, and so on) and micro (unicellular or small colonial algae and photosynthetic bacteria). We shall consider only the microscopic aquatic plants—microalgae and photosynthetic bacteria—and their potential in bioconversion to fuels, fertilisers, and petrochemical substitutes.

Microalgae in waste treatment

Large scale cultures of microscopic algae have been actively investigated for over twenty-five years⁹. Because of the high protein content of algae and the presumed large potential yields, algal research has emphasised protein production. However, practical applications of algal cultures for food or feed production are limited by high capital costs, moderate yields, poor product quality control, and lack of markets. At present, commercial production is limited to a one-ton-per-day pilot plant in Mexico producing *Spirulina* (a filamentous blue-green alga)¹⁰ and several smaller operations in the Far East which produce *Chlorella*.

The development of large-scale algal ponds for domestic and industrial liquid waste treatment has been much more successful¹¹. Although originally used only to hold wastes, ponds were observed to substantially reduce pollution indicators—biochemical oxygen demand, odour, and settleable solids. This turned out to be because pond algae photosynthetically generate enough oxygen to allow bacterial oxida-

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tion or organic wastes¹². The released nutrients (carbon dioxide, ammonia, phosphate, etc) are in turn converted into algal biomass. Design equations have been developed to relate pond area requirements to sewage strength and flows, as well as local solar insolation and climatic conditions¹³. This engineering research has resulted in the widespread adoption of algal ponds (known as 'oxidation ponds') in many countries, including the United States, Australia, Israel, South Africa, and India.

Almost all presently used oxidation ponds are the 'facultative' type—one to three metres in depth and arranged in cells of up to 50 hectares, and mixed only by wind action and perhaps recirculation of effluent. The bottom of these ponds are thus anoxic, resulting in fermentation of the settled sludge and algae, while the surface is kept aerobic by the algae, preventing odours from developing (a prime consideration in sewage treatment). The liquid detention time (days required to displace one pond volume) is long, from weeks to months. This design is optimal for waste treatment but not for algal growth. If algal biomass production is to be maximised, the ponds must be shallow (30–50 cm), have short detention times (2–4 days in summer), and be mechanically mixed (to minimise algal settling and prevent thermal stratification). These types of ponds termed 'high-rate', are much more efficient solar energy converters and can be very effective in waste treatment, provided that the algae are removed before discharge of the pond effluent.

The high algal concentrations in pond effluents represent a load of organic matter and nutrients which are regarded by regulating agencies as environmentally undesirable. High-rate ponds produce well above 100 mg l⁻¹ of algae (dry weight) and even facultative ponds normally have effluents of above 50 mg l⁻¹. These levels of suspended solids are higher than those allowed by present US waste treatment plant discharge regulations; thus, algae must now be removed from oxidation pond effluents. This step is, of course, also required for conversion of algal biomass into usable fuels.

Microalgal harvesting

Concentrating (at least 100-fold) a dilute suspension of microscopic algae is a considerable engineering problem^{14,15}. Most oxidation pond algae are below 20 µm in diameter, too small for low-cost straining, filtration, or sedimentation. Only the more expensive centrifugation and chemical flocculation reliably remove algae from pond effluents. Centrifugation requires high capital investments in large centrifuges as well as power to run them. Chemical flocculation is somewhat more cost-effective and is being installed in a few large scale oxidation pond systems in the US, but the weight of chemicals (alum, lime or polyelectrolytes) required often surpasses that of the algae harvested. The flocculated algae are collected either by sedimentation or flotation and expensive extraction steps are then needed before the algae can be used for bioconversion. Furthermore, the chemical-algal sludge is difficult to dewater and dispose of. Thus, high capital and operational costs make chemical flocculation unsuitable for bioconversion systems. Less expensive methods of removing algae from effluents are to settle them in the pond by chemical flocculation, or by prolonged (2–3-week) isolation of ponds from further sewage flows. These methods, although economical, are not suitable for algal biomass production since the algae settle on the pond bottom as a thin layer of sludge which slowly decomposes.

Water reservoirs often exhibit blooms of algae which impart foul tastes and odours to the water and which are removed by large scale 'micro-strainers'. Almost all such algae occur as long filaments or large colonies, which accounts for the effectiveness of microstrainers—rotating straining devices (Fig. 1)—in the purification of reservoir

waters. Oxidation pond algae, on the other hand, tend to occur as unicellular or small colonial types, generally under 20 µm in any one dimension, which are too small to be effectively strained. Only the sixth and seventh most common algae in oxidation ponds—the filamentous blue-green alga *Oscillatoria* and the colonial spined green alga *Micractinium*¹⁶, can easily be harvested by microstrainers. If such types of algae were normally prevalent in high-rate oxidation ponds, microalgal harvesting would no longer be a limiting economic factor in algal biomass production from liquid wastes.

Algal population dynamics

Control of algal species in ponds (whether for biomass harvesting, aquaculture, or protein production), must be based on an understanding of the specific factors affecting algal population dynamics. The following are a few generalisations about the importance of various factors. When high strength wastes (for example, food processing wastes) are serially diluted, inoculated, and incubated in the light, different biotypes will appear in the various dilutions. The most concentrated wastes will contain only anaerobic fermentative bacteria. Photosynthetic bacteria appear in the first dilution followed by a graduation of flagellated euglenoids, blue-green algae, green algae, and finally diatoms and nitrogen-fixing blue-green algae. Such successions can also be seen in outdoor ponds going from heavily loaded anaerobic ponds to the final 'polishing' or 'tertiary' ponds. Often, only one or two algal species comprise over 95% of the algal biomass in sewage ponds that are not allowed to fully stabilise (through fast dilution and heavy organic loading). A greater number of algal species is found in the more lightly loaded tertiary ponds, indicating greater stabilisation of the wastes and algal populations. The filamentous blue-green alga *Oscillatoria* is usually found at higher temperatures (being the predominant algae reported from Indian oxidation ponds) and in ponds receiving wastes low in nitrogen (such as cannery wastes). The many environmental, biological, operational and even historical factors which affect algal population dynamics in ponds interact in many ways, complicating the task of understanding algal population dynamics. For example, it is known how seeding of ponds with algae from operating oxidation ponds will affect the establishment and subsequent history of algae in the ponds. In outdoor systems, it is not possible to achieve a steady state due to constantly changing environmental conditions. Temperature, for example, is an important factor in algal successions¹⁷. The greatest problem is the lack of detailed ecological or limnological data from operating oxidation ponds. The low species diversities and rapid succession of the predominant algal species in ponds indicate a versatile system which can be affected by many environmental conditions.

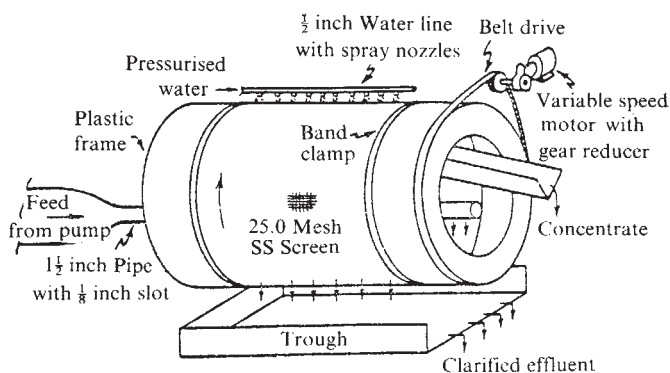
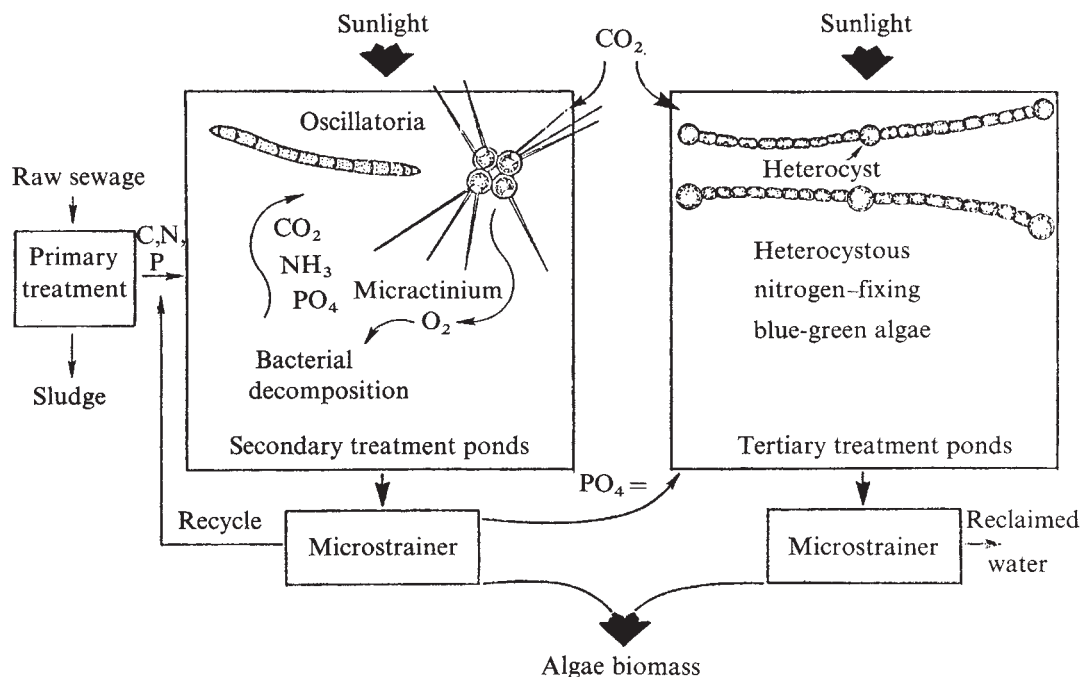


Fig. 1 Small-scale microstrainer

Fig. 2 Two-stage algal ponds for complete liquid waste treatment.



Algal species control

How, then, can algal populations be controlled so as to maintain selected algal species (or algal types) for prolonged periods? Sunlight and temperature cannot be significantly controlled, although they are critical to the succession of algal species. Pond loadings, depth, and mixing can be controlled, however; their particular effects on algal species are not yet known and would be influenced by additional environmental conditions. Thus, the selective species control methods must be adjustable in the face of changing conditions. Two such methods are presently being studied at our laboratory—size-selective recycle, and nutrient limitations.

The following is an example of size-selective recycle. In a pond operated at a five-day detention time, the predominant alga *Chlorella* is accompanied by a small percentage of *Microactinium*. *Chlorella* is the preferred food of many zooplankton such as *Daphnia*, whereas *Microactinium* is seldom grazed because its spines, which protrude in all directions (Fig. 2), obstruct ingestion by rotifers and similar zooplankton. The zooplankton cannot flourish because their population growth cannot keep up with the short detention time. If a relatively coarse screen were used to remove the *Daphnia* from the pond effluent and recycle it into the pond, a population explosion of *Daphnia* would be predicted to cause a diminished *Chlorella* population and thus lead to predominance of the undigestible *Microactinium*. Such biological control of algal species has yet to be demonstrated in ponds.

A more direct method of size-selective recycle has been used in outdoor ponds to achieve algal species control.¹⁸ It involved recycling to the pond a certain fraction of the algae harvested by a microstrainer. If a pond is heavily inoculated with harvestable species and all the algae strained from the pond are re-introduced into the pond, these algae, because they are not washed out of the pond, will have an advantage over faster-growing but non-harvestable algae. Eventually, most of the algae in the pond will be of the desired, harvestable type and the amount recycled to the ponds can be decreased. To prevent non-harvestable algae from returning, the fraction of harvested algae that must be continuously recycled is proportional to the ratio of the relative growth rates. In practice, this recycling fraction must be larger since harvesting is neither com-

pletely selective nor totally effective. Thus, the growth rate of the desirable harvestable filamentous or colonial algal species must not be too far below that of the unwanted single-cell algae. The faster dilution rates or higher algal concentrations required by cell recycling also set limits to the allowable degree of recycling. It is important to note that such recycling is a size-specific, not a species-specific control method. Other strains of harvestable algae which spontaneously appear in the ponds will be recycled and become dominant if they grow faster than the inoculated strain. Inoculations and recycling might be alternated so as to allow true species control. The principles of selective biomass recycling are also applicable to any other harvesting method (such as settling) and to other large scale microbial processes including activated sludge, fermentations, and single-cell protein production. Indeed, in the activated sludge process, the practice of returning settled bacterial biomass to the reactor results in selection for settleable bacterial flocs.

The other method now under investigation for establishing harvestable species in ponds is based on nutritional limitation. The major nutrients of algae are water, sunlight, carbon, nitrogen, and phosphorus. The degree to which any one of these nutrients is limited will affect algal populations. To maximise biomass production, sunlight should be the only limiting nutrient, since productivity is proportional to solar energy conversion efficiency. However, the final yield of algae will be determined by the limiting nutrient in the wastes, which for domestic sewage is carbon. Treatment of sewage and other liquid wastes cannot be considered complete until nutrients causing eutrophication in receiving waters (N or P) are removed from sewage treatment plant effluents, and that means adding an additional source of carbon to algal ponds. Carbon dioxide produced by a power plant or any other source could easily be brought to ponds for this purpose and would allow growth of the harvestable algae (maintained through selective recycle) up to the nitrogen algal growth potential of the sewage. An additional crop of algae could be obtained from the residual phosphate in the effluents of these ponds through further carbonation. In such a final algal production and phosphate stripping pond, only nitrogen-fixing algae could grow (Fig. 2). Nitrogen-fixing algae are blue-green and all planktonic forms are filamentous. Thus, they are easily harvested and, since growth is selective, no recycling would be required.

Wherever phosphate detergents are used, nitrogen-fixing blue-green algal biomass can be produced in large quantities (exceeding the production of the initial ponds), thereby increasing the potential N fertiliser output from the ponds to a level above that present in the wastes.

Algal bioconversion

In the multi-pond system of waste treatment and algal biomass production proposed above and indicated in Fig. 2, two ponds in series (in practice several more would be required) are used to grow successive crops of different algal types harvestable by microstrainers or similar inexpensive methods. In such ponds, energy and plant nutrients are recovered from wastes and augmented by the processes of carbon dioxide enrichment and nitrogen fixation. The most direct and practical method for conversion of the algal biomass would be methane fermentation. Direct burning is not feasible since it would require dewatering of algae, a process requiring more energy than the algae could yield.

More than one-half of the heat of combustion of the algae (10,000 BTUs per lb algae) can be converted into methane gas with digester loadings, temperatures, and detention times similar to those of sewage sludge^{20,21}. Many problems remain, including the possible resistance to degradation by part of the algal biomass, ammonia build-up in fermenters, and dewatering properties of the residual sludge. Of particular concern is the cost of conventional sludge digesters; covered anaerobic ponds might prove to be the most cost-effective large scale digestion systems. The production costs of methane would be part of the overall sewage treatment costs, making it a valuable by-product of algae-based waste treatment systems. The residues from algal digestion would contain virtually all the nitrogen and phosphorus of the algal biomass. These could be disposed of as fertiliser on agricultural land. In principle, all necessary agricultural fertiliser could be produced in this way—one acre of algal ponds providing the fertiliser required by 10 to 50 acres of modern agriculture.

The total amount of methane that can be obtained from the algal biomass produced during sewage treatment is limited to only a few percent of local natural gas needs. It has been demonstrated in laboratory studies that algae can be cultivated on the residues from algal methane fermentations.²² Therefore, by recycling the fermentation residues to the algal growth ponds algal biomass production can be expanded to the limits of available water and land. However, in such cases, no waste treatment or fertiliser production credits would help underwrite the economics of methane production. In principle, capital investments (into pond construction) and operational costs (including energy inputs) could be low enough to allow large scale algal biomass production and conversion systems. The feasibility of such large scale 'nutrient integrated' systems will depend on the solution of many technical and economic problems.

Algal productivity

So far we have emphasised the problem of algal harvesting, species control, and bioconversion because they must be solved before large scale microalgal biomass production can become feasible. However, it is the harvestable yields of algal biomass (expressed as gm per m² per day or MT per hectare per year) that determines the potential of algal systems for energy and fuel production. There is no strong theoretical basis for expecting higher yields with algae than with higher plants. Vertical shade adaptation of leaves in a crop plant is inherently a more efficient arrangement than the uniform pigmentation of algae in a mixed water column. The reflectance of sunlight from the water surface is another disadvantage of algal ponds. However, algal ponds have several compensating features, primarily the capacity for

continuously supplying all nutrients so that only light is limiting. Thus continuous culture makes possible a quick, graded response to environmental conditions and complete light absorption. The hydraulic nature of a pond system allows for simple and inexpensive operations. However, continuous cultivation also means harvesting of a dilute suspension of the microalgae. This is inherently more expensive than the periodic harvesting possible with macroalgae or land plants.

On the basis of even such a qualitative analysis, it may be concluded that rates of algal biomass production would not be necessarily higher than those observed in intensive agriculture, algal production, however, being potentially less demanding on inputs and efforts. It is not possible at present to make comparisons—many reported peak agricultural productivities are suspect on a number of accounts (methodology, border effects, and so on), and algal productivity is normally based on extrapolations from short term, small scale experiments. A figure of 50 MT per ha per yr (12 gm m⁻² d⁻¹) of dry-weight, ash-free algal production has been presented as a reasonable figure of potential productivities¹⁹. This would represent a total solar energy conversion efficiency of about 2% at 30° latitude. It might be possible to perfect algal pond systems with higher conversion efficiencies. This will require careful pond operations and genetic selection of the algal strains to be cultivated. For example, the addition of carbon dioxide to ponds could be used (in addition to the reasons listed above) to maintain an adequate pH in the ponds (it may go above 10 in the afternoons, resulting in a sharp decline of photosynthesis). This would not only extend the daily growing period, but also reduce photorespiration and the potential for photo-oxidative death²³. Another factor which could increase photosynthetic efficiencies and which may account for some of the very high yields reported is the phenomenon of photoheterotrophy. Many algae assimilate preformed organic compounds, particularly under low light intensities (as may be expected in the bottom of ponds). Since little light energy is required in the photoheterotrophic production of biomass, calculated efficiencies are high because the energy content of the liquid wastes are ignored. Controlled photoheterotrophic growth would increase the performance (load rates, organic waste destruction, and biomass production) of the primary ponds receiving settled sewage. Photosynthetic bacteria are, of course, photoheterotrophic by nature and are potentially useful in waste treatment systems. Another suggestion for improving photosynthetic efficiencies would be through decreasing the accessory pigment concentration of the algae to decrease self-shading and photoinhibition.

An important aspect to be considered is the reduction in net photosynthetic efficiencies resulting from energy inputs into pond operations such as dilution, mixing, fertilisation, harvesting and concentrating, species control, and so on. If the large scale production of algae for energy production is to be feasible, these inputs must be minimal. A slight net energy gain has been calculated from waste-grown algae using available production methods²⁴. In principle, net algal productivity should not be limited by the energy requirements of cultivation which are low; mixing at slow speeds (about 5 cm s⁻¹) is sufficient for thermal destratification and requires little energy^{25,26}. Harvesting by microstrainers is likewise not energy intensive. Water usage in pond systems is mainly through evaporation; the ability of microalgae to grow in brackish or saline waters would make it possible to use unreclaimable water exhaustively through use of a terminal evaporative pond system. Fertilisation obviously would be too expensive if mineral or synthetic fertilisers are used; nutrient recycling, whether from wastes or internally, is required. Nitrogen fixation must make up extra nitrogen requirements at a probable reduction in photosynthetic efficiencies estimated at 10%. Including energy

inputs and conversion losses, a net production of 200 M BTUs per acre per year of methane seems to be a feasible goal^{19,27}.

Future developments

The use of microbial photosynthesis for energy production is not limited to methane and fertilisers. Biophotolysis—the decomposition of water to yield hydrogen gas—has been demonstrated with algae in the laboratory^{28,29}. Heterocystous blue-green algae can be used to produce hydrogen and oxygen simultaneously in a system of horizontal glass tubes³⁰. Other algae might be capable of alternating daytime oxygen with night-time hydrogen production. Photosynthetic bacteria can produce hydrogen from organic substrates³¹, presenting a potential alternative to methane fermentation³². The production of specialised chemicals is possible. The large scale culture, of the salt-loving alga, *Dunaliella*, consisting of 80% glycerol, has been proposed (M. Aaron personal communication). Algal species containing high concentrations of fats and lipids may be used for liquid fuel production. Petrochemical feedstocks of various types may theoretically be derived from algae. However, such technologies are probably going to take longer to perfect.

Microalgal bioconversion will have a beneficial impact on environment and society as long as it does not conflict with food production or develop into gigantic centralised conversion systems. The prospects of very large scale sites (hundreds or thousands of square miles) converted to pond systems is not imminent; technological development will certainly at first favour smaller locally integrated waste treatment-algal bioconversion-nutrient recycling systems. These would be of particular value where adequate insolation is combined with a need for sanitary sewage disposal and fertiliser supplies. Such pond systems may expand, as practicable, to dispose of salt-laden agricultural drainage water by evaporation and to provide a significant fraction of local fuel needs. Energy production from microalgae might have little immediate overall impact on energy consumption of advanced industrial countries, except where favourable local situations exist. Algal bioconversion is specifically important to underdeveloped countries since such technology would

require little or no investments in scarce raw materials or complex 'hardware'. The application of sophisticated cultivation technology should be within the reach of even the poorest countries. The environmental and social impacts of algal pond systems or other bioconversion technology are expected to be minor compared with present or alternative energy sources. In the future, the developing technology of bioconversion can present mankind an alternative to depleting fossil fuels and dangerous reliance on nuclear energy.

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articles

Lateral mobility of human erythrocyte integral membrane proteins

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Fluorescein isothiocyanate-labelled integral membrane proteins are mobile in the membranes of human erythrocytes that have been fused (and haemolysed) by Sendai virus or polyethylene glycol. Minimum diffusion coefficients are of the order of $10^{-11} \text{ cm}^2 \text{ s}^{-1}$ at 37 °C. This mobility is reduced several-fold at room temperature, not detected at 0 °C, and is significantly greater in fresh than in aged blood. Mobility was assessed by observing the spread of fluorescence on labelled cells which had been fused with unlabelled cells; neither intramembrane particle aggregation nor spectrin release occurred during this process.

FACTORS which regulate the lateral mobility of cell surface components have been described for a number of cells^{1,2}, but the complexity of these cells makes it difficult to study the relevant interactions at the biochemical level. The erythrocyte membrane is more readily accessible to direct biochemical investigation but it is generally thought that lateral movements of proteins in this membrane either do not occur or are extremely limited^{3,4}. Antigenic components, visualised using direct and indirect immunofluorescent staining^{4,5} and fluorescein-conjugated lectins⁶, have not been observed to patch or cap in membranes of adult erythrocytes from various species. Similarly, the intramembrane particles exposed by freeze-fracturing are not readily aggregated in the intact erythrocyte^{7,8}. These particles represent proteins intercalated into the lipid bilayer^{9,10},

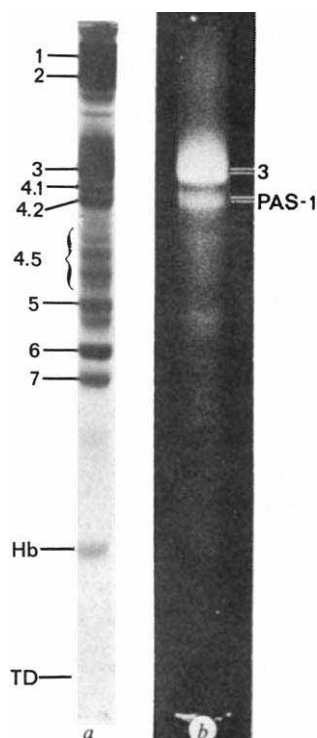


Fig. 1 A 7.5% SDS-acrylamide gel of fluorescein isothiocyanate-labelled erythrocyte membranes. *a*, Proteins stained with Coomassie brilliant blue. *b*, The same gel before Coomassie blue staining, showing fluorescent labelling. Intact erythrocytes were labelled by a modification of the method of Peters *et al.*¹⁵. Outdated human blood stored in acid-citrate-dextrose (about 3–6 weeks) was washed twice in 310 mOsm phosphate-buffered saline, pH 7.6 (PBS), removing the buffy coat after each wash, and then twice in 145 mM NaCl–10 mM NaHCO₃, pH 9.5. The packed cells were resuspended in an equal volume of a 5 mg ml⁻¹ suspension of an FITC-celite adsorbate (Sigma) in the same buffer, and agitated overnight at 4 °C. The cells were resuspended in 50 mM glycine–95 mM NaCl–10 mM NaHCO₃, pH 9.5, to stop the reaction, and layered over a $\rho = 1.13$ metrizamide shelf (32% diluted to 23.8% in the NaCl-bicarbonate buffer). After centrifugation the cells were collected from the interface, the celite having sedimented to the bottom of the tube. The cells were washed once again in the glycine-bicarbonate buffer, then twice in PBS. Ghosts were made according to Dodge *et al.*²⁵, omitting the saline and isotonic phosphate buffer steps. Gels were run according to Fairbanks *et al.*²⁶. The fluorescent gels were photographed while still in the tubes, using a long wavelength ultraviolet lamp for excitation, and a yellow barrier filter with Tri-X film. The negative was scanned with a Joyce-Loebel densitometer, and comparison of the areas under the peaks revealed that greater than 70% of the total label was present in the Band 3/PAS-1 region (>50% in band 3). No fluorescent material ran in front of the tracker dye (TD), and less than 10% of the label migrated immediately behind the tracker dye, where both free FITC and some lipids migrate in this gel system. The fluorescent band just below Band 3 on these 7.5% acrylamide gels could be readily identified as PAS-1 by its altered mobility on 5% gels²⁷.

and have been attributed to the principal erythrocyte integral membrane proteins, Band 3, and the major sialoglycoprotein^{10–14}. Peters *et al.*¹⁵, labelled a heterogeneous group of components in the human red cell membrane (both proteins and lipids were labelled) with fluorescein isothiocyanate, and measured the rate of fluorescence recovery after bleaching one half of the erythrocyte ghost. They observed essentially no recovery of the bleached half in the conditions of the experiment, implying that the labelled components, if diffusing at all were diffusing only very slowly in the plane of the membrane.

But, prostaglandin-induced changes in erythrocyte membrane lipid fluidity detected using electron spin resonance

techniques^{16,17}, paralleled by changes in membrane circular dichroism spectra¹⁸ and in the rheological properties of the blood cells^{16,18}, indicate that both lipid fluidity and protein-protein associations may play a dynamic part in the cell's response to its environment. Two recent reports indicate that surface antigens of intact human red cells can indeed undergo redistribution in the plane of the membrane. Clustering of fluorescein-labelled anti-blood group A antibodies has been correlated with a change in erythrocyte morphology from a biconcave disk to a crenated sphere²⁰, and a temperature dependent redistribution of concanavalin A (con A) bound to the cell surface, induced by anti-con A antibodies, was visualised directly on the erythrocyte surface by freeze-etching²¹. Here we report results which demonstrate a time and temperature dependent mobility of labelled proteins in the plane of the adult human erythrocyte membrane. The basic strategy of these experiments was suggested by Frye and Edidin's initial demonstration of antigen intermixing in mouse-human heterokaryons²².

Membrane labelling and cell fusion

Membrane proteins which have been operationally defined as integral^{23,24} were labelled with fluorescein isothiocyanate (FITC) (Fig. 1). After fusion (and haemolysis) of labelled and unlabelled cells with Sendai virus or polyethylene glycol (Fig. 2), the proportion of fused cells which was uniformly fluorescent increased with time except at 0 °C (Fig. 3); this is due to spreading of label over the surface of the initially bright-dark cells. Although only the end points were scored, all degrees of fluorescence distributions from bright on one half and dim on the other, to a barely distinguishable difference between the two halves, were observed.

Redistribution of label was not due to movement of fluorescently labelled haemoglobin or other cytoplasmic components because when fused lysed cells were washed in 20 mOsm phosphate buffer, pH 7.6, resuspended in 310 mOsm phosphate-buffered saline, pH 7.6, and then incubated at 30 °C (fluorescent haemoglobin was only about 1% of the total labelled protein in such ghosts) label redistribution proceeded as in unwashed cells. (This procedure was not used routinely because hypotonic lysis and subsequent resuspension in isotonic buffer resulted in shrinkage and crumpling of the membranes, making it difficult to positively identify the fused cells.) Furthermore, treatments which

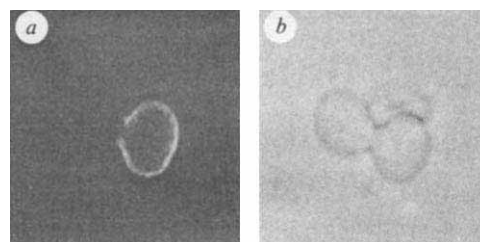


Fig. 2 An FITC-labelled cell fused with an unlabelled cell, incubated for 2 h at 22 °C. Haemolysis accompanied fusion. *a*, Fluorescence photograph. *b*, Phase contrast photograph of the same cell. $\times 1,280$. Cells were fused by Sendai virus as follows. A 50/50 mixture of a 5% (v/v) suspension of labelled and unlabelled cells in PBS was added to an equal volume of PBS containing between 5,000 and 10,000 HA per ml of egg-grown Sendai virus. This was shaken at 150 r.p.m. at 0 °C for 15–20 min to agglutinate the cells, and then at 37 °C for 10–12 min. The fusion step was terminated either by addition of an equal volume of ice-cold 310-mOsm PBS while vortexing rigorously to break up agglutinates, or by washing the cells once in about 10–15 volumes cold PBS (SS-34, 10,000 r.p.m., 30 min, 4 °C) with subsequent resuspension in an appropriate volume of PBS. As a control in some experiments, cells were fused with a 50% polyethylene glycol (molecular weight 6,000) solution by a modification of the procedure of Davidson and Gerald²⁸ for fusion of cultured cells in suspension. Fused cells which survived this process behaved as cells fused by Sendai virus.

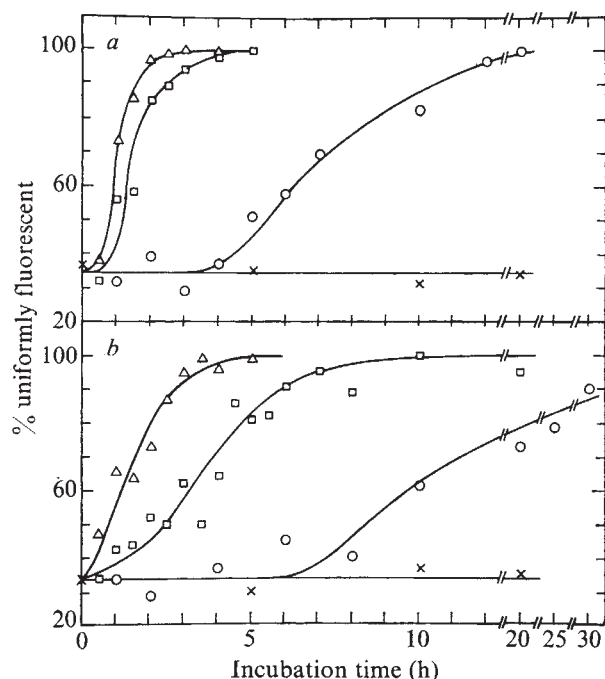


Fig. 3 Redistribition of label on FITC-labelled cells fused with unlabelled cells. Uniformly fluorescent fused cells expressed as % of the total number of fused cells which are at least partially fluorescent. *a*, Fresh blood. *b*, Aged blood stored 3–6 weeks in acid-citrate-dextrose at 4 °C. Δ , 37 °C; $\square$, 30 °C; $\circ$, 23 °C; $\times$, 0 °C. Note the breaks in the time scale at long incubation times. Cells were fused by Sendai virus as described in Fig. 2. After fusion, the cell suspensions were divided into four portions, incubated at the indicated temperatures, samples taken at various times and kept on ice until counted. To prevent bacterial growth, 0.5 mM NaN_3 was routinely included during the incubations; curves for redistribution of label were identical in the presence and absence of azide. Fused fluorescent cells were scored as uniformly fluorescent, that is, no difference in intensity was distinguishable on one half of the fused cell relative to the other half, or as not uniformly fluorescent, that is, some difference was visible. A total of 100 cells from each sample were scored for the determination of each point; points from one representative experiment are plotted. Note that at 0 time one third of all fluorescent fused cells are uniformly fluorescent; these result from the fusion of two initially fluorescent cells with one another. This is the expected outcome of a fusion process in which it is equally likely for any cell to fuse with any other and in which only fluorescent fused cells are counted.

remove the so-called 'peripheral' proteins^{23,24}, such as low ionic strength extraction, high salt washes, *p*-chloromercuribenzoic acid, or 10% acetic acid treatments^{24,29}, failed to elute any fluorescence from ghosts or fused cells. Spreading of label is also not due to movement of dye molecules which have simply adsorbed on to or partitioned into the membrane, since less than 10% of the total fluorescent label migrates in the position of free fluorescein isothiocyanate on sodium dodecylsulphate (SDS)-acrylamide gels (Fig. 1). In addition, FITC-celite pre-incubated in 50 mM glycine–95 mM NaCl–10 mM NaHCO_3 , pH 9.5, did not label the cells, and all cells were washed in this buffer after labelling (Fig. 1).

Fusion was always accompanied by haemolysis. But, neither proteolysis nor extensive intramembrane particle aggregation could be detected in Sendai virus-treated erythrocytes. SDS-acrylamide gels of haemolysed and fused cells incubated at 37 °C until label had become uniformly distributed were identical to gels of ghosts from untreated cells (Fig. 1) in terms of fluorescent labelling and staining with Coomassie blue. SDS-gel electrophoresis is sufficiently sensitive to detect proteolysis of ghost membrane proteins by microgram per millilitre quantities of trypsin^{30,31}. Moreover, proteolysis leads to vesiculation of the ghosts³⁰ and

clustering of the intramembrane particles¹⁴, which were not observed after treatment by Sendai virus. Samples of the supernatant and the pellet of cells fused and incubated in the same buffer without resuspension were electrophoresed in parallel. Supernatants to which was added an amount of spectrin (bands 1 and 2) and actin (band 5) corresponding to less than 10% being released from the cells into the lysate provided a standard for comparison, and demonstrated that the treatments used in our experiments did not result in detectable spectrin (or actin) release from the membrane. It was important to rule this out, since the ability to induce redistribution of the intramembrane particles has been correlated with previous treatments which dissociate the peripheral membrane proteins spectrin (and actin) from the cytoplasmic surface of the membrane⁸. In addition, curves plotted for label redistribution in polyethylene glycol-fused cells (not shown) correspond closely to those for Sendai virus-fused cells (Fig. 3).

Membrane protein mobility

The abrupt increase in uniformly fluorescent haemolysed and fused cells after a short lag time is particularly striking at physiological temperatures. Decreasing the temperature to 22 °C dramatically slows the rate of label redistribution, and further reduction of temperature to 0 °C prevents it completely. It is evident from the curves obtained that some fused lysed cells become uniformly fluorescent sooner than others. This heterogeneity is most clearly visible at lower temperatures where the rates of label movement are slower. Evaluation of variations in the rate of label movement at physiological temperatures is difficult, due to the errors involved in determining rates by scoring a population of fused lysed cells for each time point. Direct determination of rates on individual fused cells by measuring the increase in fluorescence intensity on the initially dark half of the fused cell would clarify this question.

Approximate diffusion coefficients for minimum rates of label redistribution have been calculated (Table 1). Using fresh blood, Peters *et al.*¹⁵ set an upper limit on the diffusion coefficient for fluorescein isothiocyanate-labelled components in the erythrocyte ghost membrane at $3 \times 10^{-12} \text{ cm}^2 \text{ s}^{-1}$ at 22 °C, which compares favourably with our value of $6 \times 10^{-12} \text{ cm}^2 \text{ s}^{-1}$. The diffusion coefficients which we calculate at 37 °C are about an order of magnitude greater, however, and approach those determined for plasma membrane proteins in other cell systems, which fall between about 10^{-11} and $10^{-10} \text{ cm}^2 \text{ s}^{-1}$ (refs 33–38). The possibility that the fusion and lysis process itself affects the lateral

Table 1 Minimum diffusion constants determined for FITC-labelled integral membrane proteins in the erythrocyte

Temperature (°C)	Fresh blood ($\text{cm}^2 \text{ s}^{-1}$)	Aged blood ($\text{cm}^2 \text{ s}^{-1}$)
37	4×10^{-11}	2×10^{-11}
30	3×10^{-11}	1×10^{-11}
23	6×10^{-12}	2×10^{-12}
0	No movement of label observed	

Approximate diffusion coefficients for the integral proteins of the slowest cells to show complete label redistribution were determined from the data in Fig. 3. The formula developed by Huang³² for diffusion from one half of a spherical shell into the other was used, assuming first that the time course of label redistribution on these cells can be approximated by such an exponential decay process, and second that the fused cell doublet was a sphere with a surface area twice that of a single cell (verified by measurement on photographs of fused cells). We further assumed that the time for label equilibration on the slowest cells was the time at which at least 90% of the initially bright-dark fused cells had become uniformly fluorescent, and that such fused cells would appear uniformly fluorescent to the human eye when twice the relaxation time for label equilibration in a fused doublet had elapsed (compare Fig. 2 in ref. 32).

mobility of the labelled proteins cannot be ignored. It is significant, however, that the diffusion coefficient inferred for membrane antigens at 37 °C in mouse-human heterokaryons produced by fusion with Sendai virus^{22,23,32} is within the range of values we have found in fused red cells. A pronounced dependence on relatively small variations in absolute temperature seems to be characteristic of the lateral mobility of membrane proteins^{22,39}, in contrast to the far more rapid diffusion of membrane lipids, which is relatively unaffected by corresponding variations in temperature³³⁻³⁸. Factors such as membrane viscosity, phase transitions and separations of the membrane lipids, boundary lipids associated with a particular protein, and dynamic interactions with other proteins, within or beneath the bilayer², almost certainly regulate the lateral mobility of these membrane proteins.

Table 2 Effect of an ATP regenerating system on the rate of redistribution of label

Time of incubation (min)	% Fluorescent fused cells uniformly fluorescent			
	Fresh blood		Aged blood	
	+ATP	-ATP	+ATP	-ATP
0	36	36	32	32
30	40	36	—	—
60	75	52	44	32
90	92	68	64	44
120	100	98	84	52

Freshly drawn blood was washed thoroughly and aged to reduce intracellular ATP levels by incubation at 37 °C for 16 h in 135 mM NaCl, 20 mM NaHCO₃, 3 mM KCl, 2 mM MgCl₂, 1 mM CaCl₂, and 200 µg ml⁻¹ benzylpenicillin, pH 7.6 (ref. 41), and then labelled as for Fig. 1, simultaneously with freshly drawn blood from the same donor. Cells were fused with Sendai virus as for Fig. 2, the suspensions were divided into two portions and washed once in 310 mOsm PBS, pH 7.6, as described in Fig. 2, and then resuspended in either PBS (-ATP), or PBS plus 1 mM ATP, 2 mM MgCl₂, 5 mM phosphoenolpyruvate, 20 µg ml⁻¹ myokinase, 50 µg ml⁻¹ pyruvate kinase, pH 7.6, (+ATP), and incubated at 37 °C as described in Fig. 3. The omission of either phosphoenolpyruvate or ATP from the regenerating system gave results which were not significantly different from those in PBS alone.

The marked differences in rates of label equilibration for fused lysed cells made from fresh blood as compared with aged, ATP-depleted¹⁰ blood suggested that intracellular ATP levels might affect the lateral mobility of the membrane proteins. Incubation of the fused lysed cells in the presence of an ATP regenerating system increased the rates of label redistribution significantly (Table 2). Since cells from both aged and fresh blood are invariably haemolysed during fusion and are thus equally depleted of metabolites, and since the presence of an ATP regenerating system does not decrease the length of time for label equilibration to equivalent values for both fresh and aged cells, factors in addition to energy availability probably control the lateral mobility of these membrane proteins. Cell shape, deformability, and divalent cation content change dramatically during cell ageing and ATP depletion^{40,41}, and may have an important role in these complex phenomena.

Discussion

Observations of parameters influencing the lateral distribution of the intramembrane particles exposed by freeze-fracture have led to the notion that lateral movements of the integral membrane proteins are restricted by a spectrin-actin meshwork beneath the erythrocyte membrane, either by a direct linkage mechanism or by trapping between the interstices of the meshwork^{8,24,42}. As pointed out before⁸, however, aggregatability of the intramembrane particles must be distinguished from direct measurements of protein

mobility. The importance of distinguishing between particle aggregation and mobility becomes evident if it is borne in mind that treatments which induce particle aggregation perturb the membrane. Such perturbations may alter dynamic interactions between proteins so as to favour associations that restrict mobility.

Moreover, although the intramembrane particles seem to consist, at least in part, of Band 3 and the principal sialoglycoprotein¹⁰⁻¹⁴, they may not coincide with the fluorescently labelled proteins whose mobilities we observe at a light microscopic level. Our data do not exclude the possibility that some of the proteins in the erythrocyte membrane are less free to diffuse than others, since the rates of label movement which we observe represent an average, both over a population of cells and a population of integral membrane proteins. But, if all the label present in the Band 3 region (at least 50%, see Fig. 1) were to be immobilised with respect to the other labelled components, we would expect that at the end point, the population of fluorescent fused cells would consist of approximately 33% uniformly and brightly fluorescent cells and 66% in which three-quarters of the label is on one half of the fused cell and one-quarter on the other half. This is not the case. A report that the rotational diffusion of Band 3 proteins (labelled with eosin isothiocyanate) is not affected by removal of most of the spectrin from erythrocyte ghosts⁴³ is additional evidence that protein mobility and particle aggregation are controlled by different factors. Specific and dynamic interactions between spectrin⁴⁴, or other peripheral membrane proteins, and the integral membrane proteins may regulate the lateral mobilities and associations among proteins in the plane of the membrane. This could provide a potential mechanism for the dynamic interaction between extracellular events and internal metabolic processes in the erythrocyte.

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Plate linkage mechanism to account for oroclinal deformation in the Western Cordillera of North America

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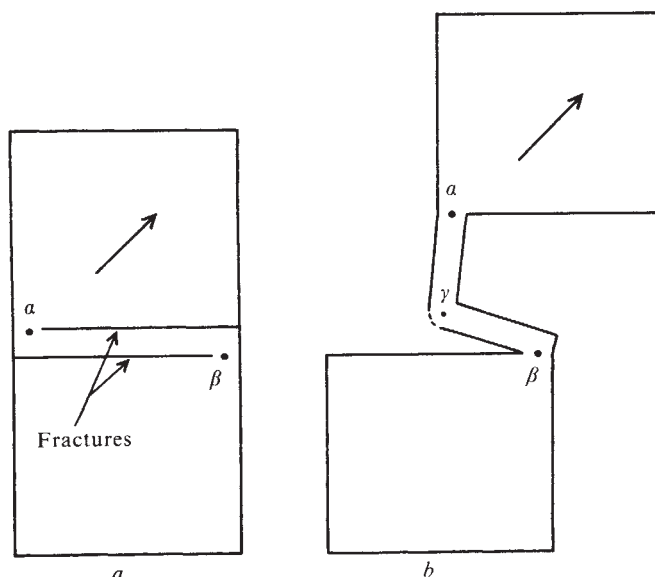
Plate tectonic theory is used to predict the motion of small plates which remain coupled within discrete regions to adjacent large plates. Taking the Western Cordillera of North America as an example, it is shown that the three oroclines situated between Vancouver and Northern California are very largely removed when that portion of the orogenic belt south of the Klamath mountains is rotated back in time with the Pacific plate while retaining the northern Cordillera as a rigid portion of the American plate. The rotation at the Pacific–America pole is found to be 14.4° which suggests that the deformation began during the Oligocene.

PLATE boundaries are generally well defined, both seismically and topographically. When traced out around the globe they are seen, in most cases, to divide the surface into a number of discrete plates each decoupled from its neighbours. There are, however, regions where the boundaries cannot easily be delineated, for example, in parts of the Mediterranean or the southerly continuation of the East African Rift. There may be various reasons for the uncertainty including, in some instances, a lack of seismicity. There is also the possibility, however, that some of these regions are more complicated than is implied by a simple plate tectonic interpretation. Wilson² envisaged plate boundaries to be of three types which are currently referred to as spreading ridges, subduction zones and transform faults. Thus a ridge may change or be 'transformed' into a fault or subduction zone at what he called a transform. To these three we may add a fourth type of boundary where the plates become attached to one another. Clearly this situation can only exist very briefly because of the rapid build-up of stresses induced by the relative motion between the plates. Such a situation must have existed in Baja California when this portion of the North American plate was transferred to the Pacific³. Here the outcome was relatively straightforward and with new transform faults and intervening ridge segments developing to the east of Baja to accommodate the strain. In other similar instances an alternative process may sometimes have occurred whereby the two original plates remain joined by a sliver of lithosphere which is hinged to each. Consider for example, the plate shown in Fig. 1a which is being split apart by rifting. If instead of a single fracture, there develop two major fracture zones, neither of which is able to completely truncate the plate. Motion of the northern half in a north easterly direction pulls the sliver away from both the northern and southern plates. Assuming the sliver to remain rigid except in the vicinity of the hinging points, then finite motion cannot occur until a third hinge pole labelled γ in Fig. 1b becomes established somewhere between the other two. For any defined finite rotation between the two major plates, there must exist a unique solution to the orientation of the small 'linked' plates.

Over 20 years ago S. W. Carey noticed that, while some orogenic belts are dominantly linear in plan view, others show sharp inflections⁴. The question which he posed then and which remains today is whether such bends were part of the orogenic belt at the time it was forming or whether they formed later hence representing what he called 'an impressed strain'. He assumed the latter interpretation to be correct and defined the term orocline for such bends. We may see how the concept of hinging small plates to large ones can explain the formation of oroclines by considering a hypothetical linear orogenic belt labelled ABCD in Fig. 2a.

The northern portion A is kept fixed and the southern portion D is allowed to move with respect to A. The pole AD is placed at various points on the stereogram and in each case the rotation angle of D to A is 20° . To accommodate this rotation without rupturing the orogen, two link plates are permitted to form, namely B and C. The kinks which subsequently appear, due to the rotations, are shown in Fig. 2, b–f. The poles AB and AD are fixed with respect to the stereogram while BC and CD are instantaneous. The latter two poles may, however, be located since the distances between the hinge poles are known. Poles AC and BD are instantaneous with respect to the stereogram and to all four plates. They may be located by graphical means using the

Fig. 1 One of several ways in which linked plates may form. *a*, The northern portion of the plate begins to pull away towards the north east causing fractures. These fractures do not sever the plate but instead leave the fractured central portion joined at α and β . *b*, With finite motion the distance α to β decreases causing the interconnecting plate to buckle at a third point γ .



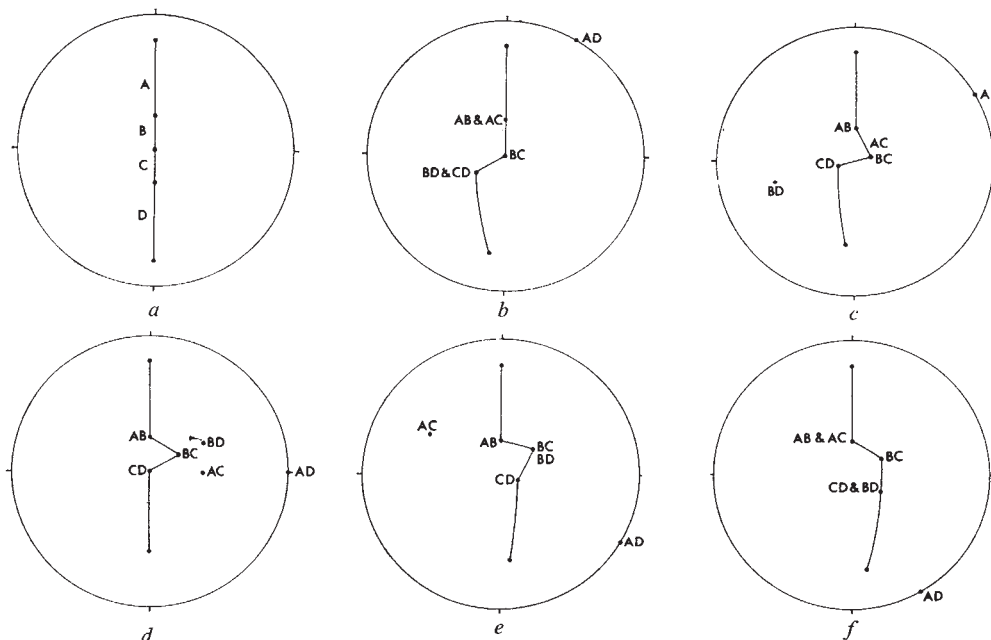


Fig. 2 Diagram illustrating how different oroclinal configurations may develop depending on where the pole of relative motion for the driving plates is located. *a*, Initial condition with linear orogenic belt ABCD. *b-f*, Oroclines generated by placing the pole AD at five different points on the great circle which forms the equator of the Lambert equal area projection.

property that the three poles for any triple plate problem always lie on a great circle. Thus considering, for example, the pole BD in Fig. 2; this occurs at the intersection of the two great circles passing through first CD and BC and also that through AB and AD. Not only are the locations of poles BC, CD, AC and BD instantaneous, but for a given constant rate of pole AD, rates at all the other poles vary continuously.

Carey realised that oroclinal configurations do not usually occur in isolation and grouped them together in pairs⁵. In doing so, he recognised left lateral and right lateral shear as the cause and defined the structures as being sinistral and dextral coupled oroclinal configurations respectively. In applying the plate tectonic interpretation outlined above, we see that it is impossible for rigid plates to rotate about two hinge poles and it follows that either the lithosphere is not behaving rigidly in these conditions or else the oroclinal configurations should occur in sets of three. Where three oroclinal configurations are present, the terms dextral and sinistral are inappropriate, however, it is apparent that the deformation shown in Fig. 2 could have proceeded in such a way that the hinge pole BC moved out of line towards the west rather than to the east.

The Cordillera along the western margin of North America show several sharp inflections in the trend of both the orogenic belt and the outcrop pattern of the associated granitic intrusions. These bends have been interpreted as being due to dextral shear by several authors including Carey (refs 5-7). The evidence in support of these bends being secondary is from palaeomagnetism and from various branches of geology. Palaeomagnetic results from the Siletz River volcanics in Oregon⁸ and the Columbia Plateau basalts⁹ all show a clockwise rotation subsequent to their formation. The geological evidence includes the trend of the structural grain of the rocks which is seen to follow the outcrops of the orogenic belt and is hence deflected through large angles at the oroclinal configurations. Other evidence comes from the turbidites of the Tyee formation in Western Oregon which in their present configuration, are seen to have flowed northwards into an oceanic embayment in the continental margin rather than to the west into the Pacific¹⁰.

An examination of the geological map of North America shows the orogenic belt to be dominantly linear between latitudes 60°N and 52°N. Near Vancouver Island however, there is a sharp bend which deflects the belt along with the granite plutons through approximately 30° and this is taken to be the third orocline which, along with those

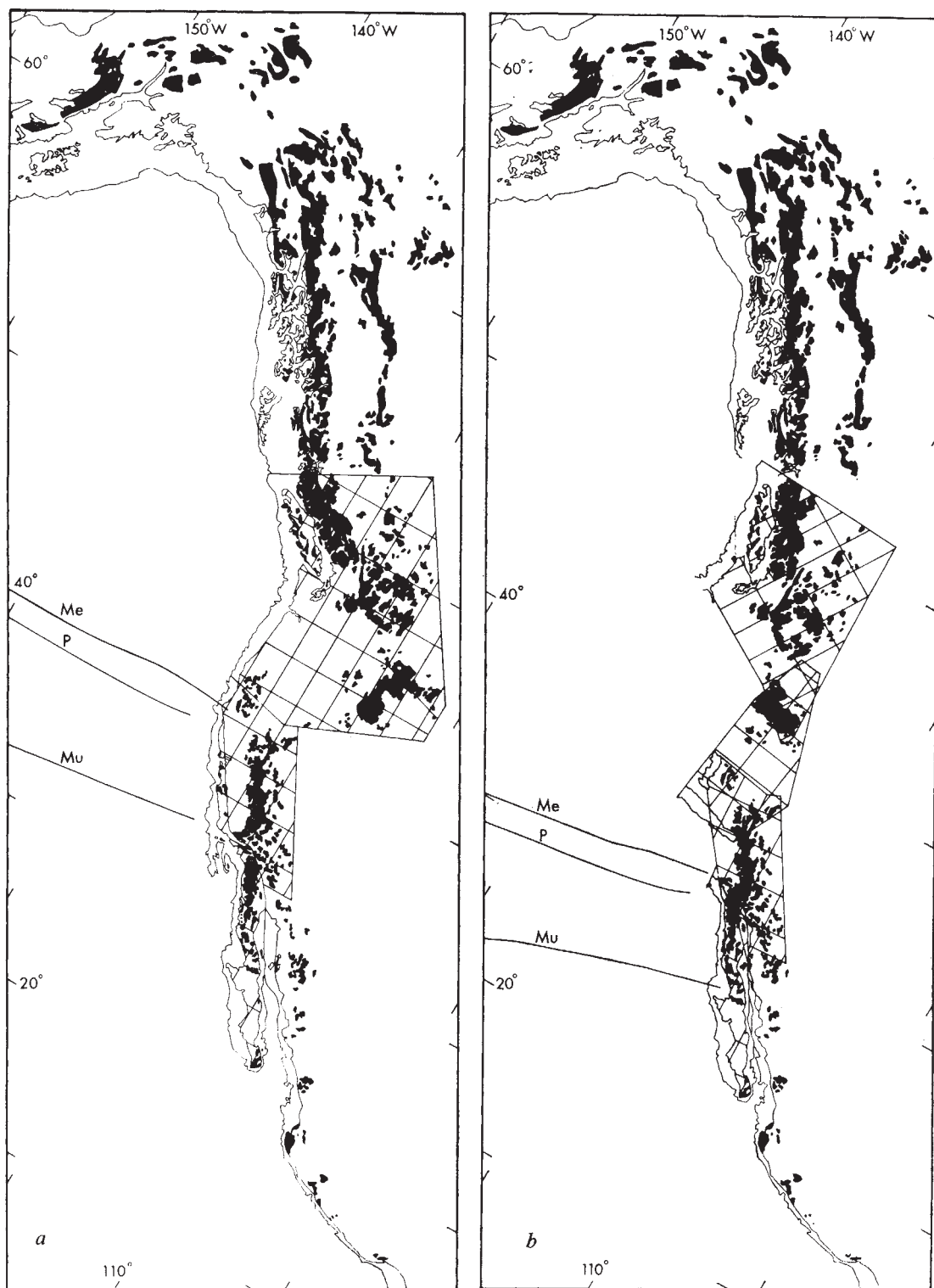
identified by Carey⁵, is required in order that these bends may have developed by linkage of rigid plates. Further south the orogen is seen to bend in Idaho from a NW to SE trend between Vancouver and Idaho to almost NE to SW between Idaho and the Klamath Mountains. South of the Klamath Mountains the belt apparently runs in a SSE direction parallel to the Sierra Nevada; however the late Tertiary volcanic rocks in the Cascade Mountains and the sediments in the Great Valley may conceal the fact that relative motion has occurred between these two regions during or after the period of oroclinal deformation.

If the bends are accepted as oroclinal configurations then the driving mechanism can only have come from the relative motion between the Pacific and American plates. It is envisaged that a small portion of the latter became rigidly attached to the Pacific plate in mid Tertiary times when that segment of the Farallon plate between the Pioneer and Murray fracture zones was completely consumed in the subduction zone³. The process was probably essentially similar to the incorporation of Baja California into the Pacific plate but in this instance the relative motion did not enable this piece of the Cordillera to become completely sheared off from America. Instead, the compression was transmitted all the way up the orogenic belt as far as, or even past, Vancouver and it was forced to buckle at the three weakest points. Once these kinks or hinge poles became established, then it should have been possible for the deformation to proceed in such a fashion that the segments of the Cordillera in between remained rigid. The edges of these segments should then have followed the rules of plate tectonics from which we may predict the nature and amount of compression and/or extension between them and the American plate.

In accordance with Carey's practice of giving names to the oroclinal configurations, I shall refer to the hinge poles in order from north to south as Vancouver, Idaho and Klamath respectively. Unlike other poles of relative motion which are merely the geometrical consequences of the motion of rigid plates, hinge poles have considerable geological significance since they represent the sites of profound tectonic activity. The linked plates in between these poles will be referred to as the Washington plate north of the Idaho hinge pole and the Oregon plate between the Idaho and Klamath poles.

To restore the orogen to its original shape, the required parameters are the coordinates of the three poles and the coordinates and rotation angle of the pole of finite relative

Fig. 3 The western margin of North America showing: *a*, the present configuration of the proposed linked plates and *b*, the Mid Tertiary (approximately 30 Myr ago) orientation of these plates as predicted by the model described in the text. Areas shaded in black are Mesozoic granitic intrusions. Fracture zones on the Pacific plate are Me, Mendocino; P, Pioneer and Mu, Murray.



motion between the Pacific and American plates for the period in question. The locations of the poles between the linked plates have been estimated from the tectonic map of North America¹¹ and are listed in Table 1. The youngest magnetic anomalies between the Pioneer and Murray fracture zones vary from 10 in the north to 6 in the south³. This suggests an age of between 32 and 26 Myr BP as the time of onset of oroclinal deformation while the opening of the Gulf of California 4 Myr ago¹² may be taken as marking the end of that deformation. The pole and angle for the finite motion during this period cannot be determined directly and indirect methods using circuits involving perhaps five or more plates lack precision¹³. For

these reasons, I shall use the best available data for the instantaneous relative motion¹⁴. Even more uncertain than the location of the pole is the rotation angle for the period. Molnar and Atwater have shown, using plate circuits, that the instantaneous rate was significantly slower in mid Tertiary times than at present and their reconstruction for 29 Myr ago suggests that a rotation of about 14° has occurred between then and the beginning of the opening of the Gulf of California. Instead of using this angle, we may proceed to determine the rotation required to line up the three hinge poles on a great circle, this being the maximum possible angle as further rotation would require the orogenic belt to have been longer before the oroclinal

Table 1 Estimated rotations taken from maps compared with predicted values using the proposed plate tectonic model

Plate pair	Lat.	Pole	Long.	Rotation angle (°)	
				Estimated	Predicted
America–Washington	51.0°N		127.0°W	30	31.4
Washington–Oregon	45.5°N		115.0°W	110	114.0
Oregon–Pacific	42.0°N		123.5°W	80	70.8
Pacific–America	52.0°N		76.0°W*	14†	14.4

*This pole was determined by Chase and is quoted in ref. 14.

†This angle has been determined by the writer using Chase's pole and the 29 Myr b.p. reconstruction in ref. 13.

deformation than it is at present. A computer program was written to determine the locations of the hinge poles and the amount of rotation at these poles for any specified rotation at the Pacific–American pole. Using this it is possible to determine not only the angle required to line up the orogenic segments but also to examine the intermediate stages during the deformation. It was found that 14.4° of rotation about Chase's pole¹⁴ was required to line up the three hinge poles; the rotations which are incurred at these poles are shown in Table 1. It is geometrically possible to rotate the Klamath pole about any arbitrary point on the globe to line up the three hinge poles on a great circle. However, such rotation would not, in general, restore the whole orogenic belt to a great circle configuration. Theoretically, there exists a great circle which is the locus of the Pacific–American pole which satisfies the condition that the orogen be straightened out in the suggested manner. The close comparison of estimated and calculated values for the rotation listed in Table 1 tends to confirm that Chase's pole lies on, or is at least very close to, this locus. The distance along the locus from the orogen determines the amount of rotation required and here again, there is a close resemblance between the calculated value of 14.4° and that estimated from the published reconstruction¹³. This shows that if the pole of finite motion lies close to the present instantaneous pole, then it is to be expected that with an initial great circle configuration, the Cordillera would have been distorted into almost exactly the shape we see today during the allotted time span of about 25 Myr.

Before making the reconstruction which is shown in Fig. 3, estimates of the boundaries of both the Washington and Oregon plates had to be made. These must, to some extent, be tentative, however, moving them to a limited extent will not seriously modify the tectonic implications of the model. Perhaps the most critical boundary is the one between that part of California which was moving with the Pacific, and its contact with the American plate. It is not obvious from the geology where this boundary should be placed but it must have been to the east of the Klamath Mountains in northern California. It is unlikely to have been to the east of the Sierra Nevada for the whole period of deformation as this would have opened up a substantial gulf further south in a similar fashion to the southern end of the Gulf of California. Some motion between the Sierra Nevada and the Basin and Range province is here assumed to have occurred, however, as this can account for the late Tertiary dilation and consequent faulting in this region. By analogy with the present San Andreas transform fault, it is likely that the boundary in question was also a fault and this may now be largely concealed by the sediments of the Great Valley. The most likely location, however, seems to be in the metamorphic belt of palaeozoic and mesozoic strata on the north-western flanks of the Sierra Nevada batholith. Here faults with a suspected strike slip component¹⁵ may be traced over 300 km from the Cascade Mountains in the north to the eastern margin of the Great Valley where presumably they continue under the late Tertiary sediments.

We may now examine the nature of the relative motion between the linked plates and the American plate in greater detail by dividing up the total rotation at the Pacific–

American pole into 24 increments of 0.6°. In doing so, each increment represents approximately 1 Myr. The cumulative rotations at the hinge poles are shown in Fig. 4 while in Fig. 5, lines joining these poles at every third increment are superimposed on a simplified geological map of the area. It can be seen that the rates of rotation decreased at all three poles with time from which it follows that the geological processes associated with these rotations must also have varied accordingly. If we take, for example, the compression along the Rocky Mountains between the Washington plate and the American plate, it is found that about 90% of the total compression was accomplished during the first half of the period. From the migration path of the instantaneous pole between the Oregon and American plates (see Fig. 5) it is predicted that initially there would have been compression along the entire boundary between the two. The early compressive phase at the southern end may at present be represented on the American plate by the belt of metamorphic rocks to the west of the Sierra Nevada referred to earlier. The outcrops show a bend of about 25° at latitude 37°N which if due to oroclinal deformation could be interpreted as being caused by the initial high rate of clockwise rotation of the Oregon plate relative to the Pacific. With time the nature of the motion on the Oregon–American plate boundary changed with extension beginning in the south and migrating northwards. During the last half of the period it is predicted to be dominantly extensional throughout and it is envisaged that at this time the Sierra

Fig. 4 Cumulative rotations at the three hinge poles as predicted by the model. The slope at any point on these curves gives the instantaneous rate in relation to the American–Pacific rate. Curve *a*, Idaho pole; *b*, Klamath pole; *c*, Vancouver pole.

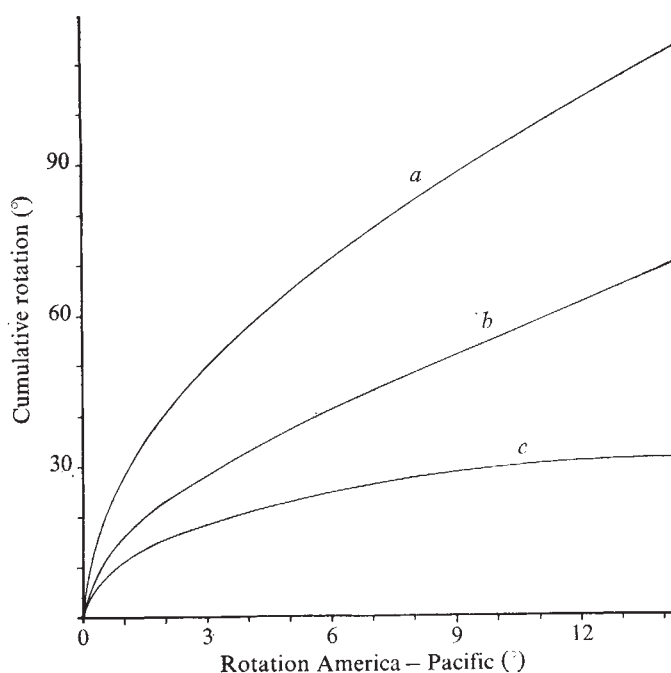
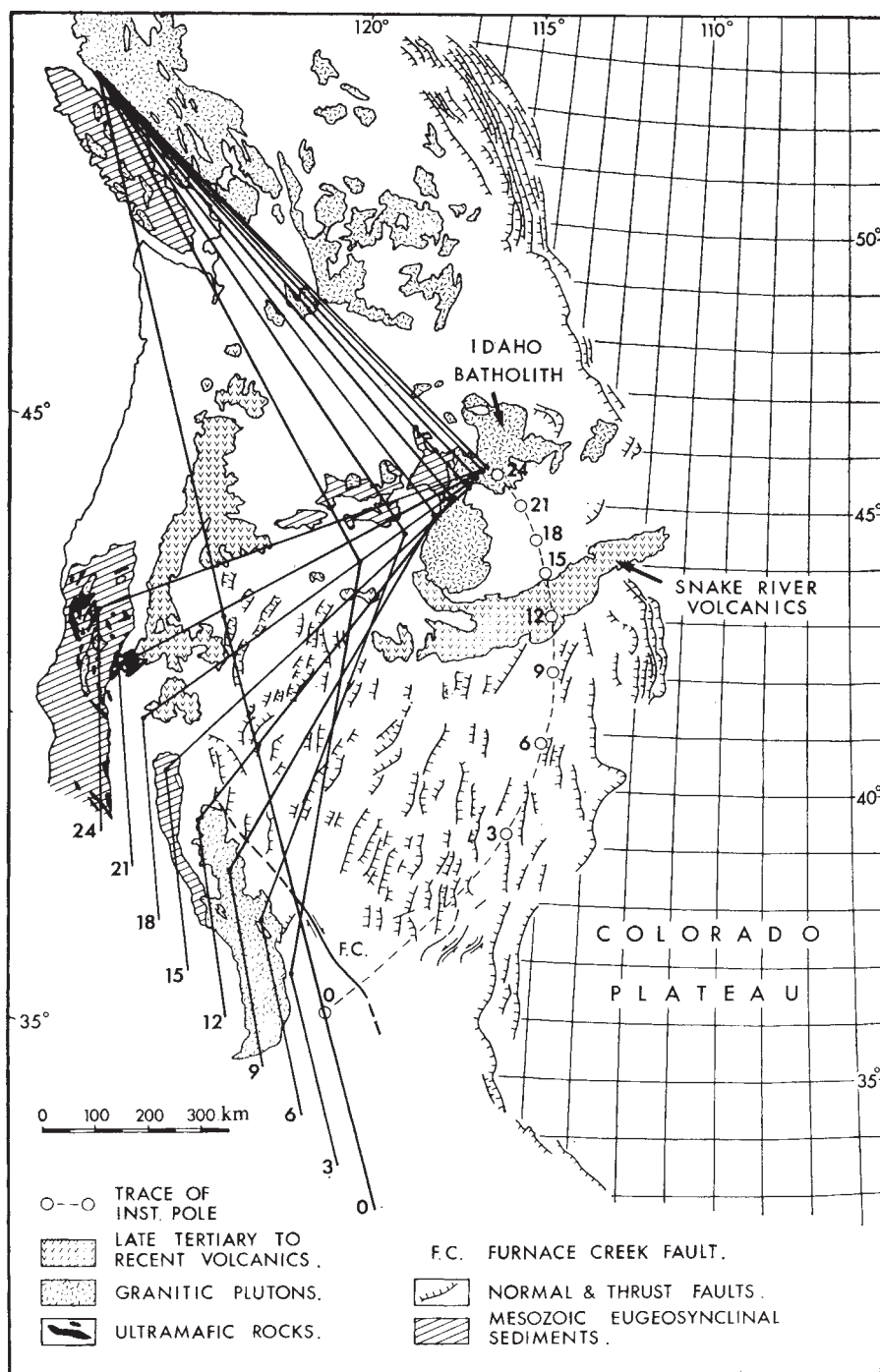


Fig. 5 Simplified geological map of the region showing the predicted motions of the linked plates and hinge poles in relation to the American plate. For clarity only a third of the 24 increments which were used to construct Fig. 4 are shown and each therefore represents a rotation of 1.8° between the American and Pacific plates. The trace of the instantaneous pole across the Basin and Range province is that for the motion between the Oregon and American plates.



Nevada region moved with the Pacific plate. In doing so, it would have moved north-westwards relative to the Basin and Range and it is likely that most of the motion was taken up on the Furnace Creek fault. Tension was generated in two distinct areas at this time namely, the Great Basin (north of latitude 36°N) and the Southern Basin and Range (between latitudes 36° and 28°N). In the former case, the relative motion was controlled by the Oregon-American pole and there is some indication that the normal faulting in this region has a radial distribution about a point in central Idaho (see Fig. 5). The implied degree of extension is very large, particularly on the western side of the Great Basin, from which I conclude that, in addition to the visible faulting, some of the dilation must be hidden under the vast areas of late Tertiary volcanism. In particular, the rifting and volcanism in the vicinity of the Snake River may be attributed to the final phases of

deformation. Here instead of the extension being distributed over a broad zone as it is elsewhere, it became very much more localised. This may simply be due to this region being so close to the pole of relative motion. If the Snake River downwarp was initiated at the time that the instantaneous pole was crossing it, then we should expect the volcanism to appear first in the west where the extension rate was highest. The time of this initial rifting would have been about 15 Myr before the present according to the model which compares favourably with a radiometric age of 13 Myr for the oldest Snake River rocks¹⁸. As the pole migrated further north, so the more easterly portions of the rift would have opened up and hence we can account for the observed age progression from west to east with time¹⁶. The other region undergoing extension at this time lay to the south of the Sierra Nevada. Assuming the Sierra Nevada to have moved with the Pacific plate for say half

of the total rotation, then the extension between this and the American plate would have amounted to about 400 km. The Southern Basin and Range may be traced in a NW-SE direction over a distance of about 1,200 km so here again considerable dilation is predicted. If there is sufficient evidence to show that this is too high then we may be led to assume that the motion of the Sierra Nevada with the Pacific plate occupied a smaller proportion of the total rotation between the Pacific and American plates.

The validity of the proposed mechanism will ultimately depend on how well it can explain all the large scale Tertiary tectonic phenomena in the Western Cordillera of North America. The mechanism should also be tested in other areas, particularly where palaeomagnetic data suggest that large rotations have occurred during relatively brief periods of geological time. In some instances oroclines may not be observed as they require the existence of an orogenic belt in the vicinity of the hinging poles. While linkage may perhaps be favoured in tectonic regimes where the crust

has a strong grain, there does not seem to be any reason why it should not also be found to occur elsewhere.

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letters to nature

Uranus rings: an optical search

THE discovery of rings surrounding Uranus¹⁻⁴ has prompted a careful re-examination of telescopic observations made nearly a year ago with a new type of solid-state area detector called a charge-coupled device or CCD. The extreme narrowness of the individual Uranus rings explains why they had not previously been detected either by visual or conventional photographic methods, for even if they were composed of highly reflecting, ice-coated particles, the rings would likely be hidden in the glare of reflected light from Uranus itself. The average reflectivity (averaged over the disk) of Uranus in the blue and green regions of the spectrum is very high, $\sim 0.55-0.60$. At longer wavelengths, however, methane in the planet's atmosphere begins to absorb much of the incident solar radiation, hence the bluish-green appearance of Uranus in the telescope. In the deep methane absorption band at 886 nm the reflectivity of the planet drops to only 0.012, and Uranus would thus appear very dark to any detector which was sensitive to this near-infrared region of the spectrum. It is of particular significance to the ring detection problem that such imaging would suppress the light reflected from the planet relative to that reflected from any nearby solid bodies, such as satellites or ring particles, by a factor of nearly 50. Unfortunately, most detectors have lost nearly all of their detectability at wavelengths longer than 800 nm.

The CCD is an exception. Low noise and quantum efficiencies of 0.5 and higher permit telescopic imaging of Uranus in the 886 nm methane band with acceptably short exposure times. In May 1976 we obtained several dozen images of Uranus in this band using a CCD camera at the f/13 cassegrain focus of the University of Arizona's 154 cm (Mt Lemmon) telescope. Exposure times ranged between 1 and 5 min. These images, recorded digitally on magnetic tape, have recently been re-examined to see if they contain any hint of the Uranus rings. Because the widths of the individual rings¹ lie way below the resolving power of any groundbased telescope, we can evaluate only an average reflectivity based on the simplifying assumption that all of

the ring material is spread uniformly between the innermost and outermost rings—a region of space extending from 44,000 to 51,000 km from the centre of Uranus. Evidence of the rings has not been found in any of the CCD images. But it is possible to set an upper limit of approximately 0.0001 for the average reflectivity as defined above.

A preliminary examination of the reported occultation events implies that these narrow rings occupy $\sim 2\%$ of the space between the innermost and outermost events, and an analysis by Hubbard indicates that the individual rings themselves are only partially (approximately one-half) filled with solid particles⁵. If these numbers are accepted as being essentially correct, we find that the average reflectivity of the individual particles must be at most a few per cent, much closer to that of carbonaceous chondritic material than to the ice-coated particles in the rings surrounding Saturn⁶⁻⁸. Considering the extremely low temperatures characteristic of this remote part of the solar system (<90 K), this preliminary conclusion on the nature of the Uranus ring particles comes as somewhat of a surprise.

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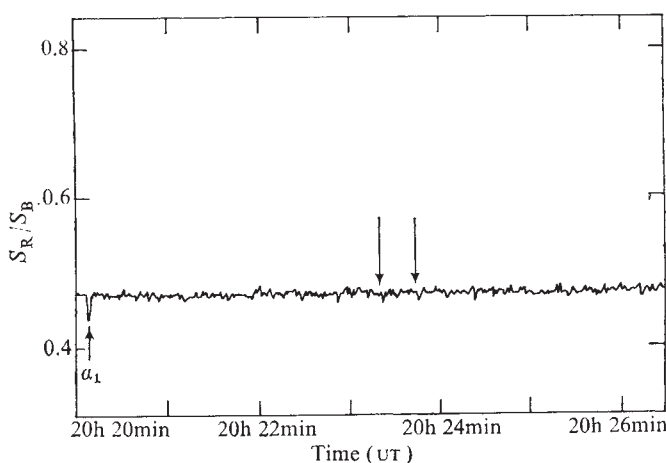
Observations of Uranus occultation events

A PRELIMINARY analysis of photometric data obtained by Zellner at the Perth Observatory during the Uranus appulse to SAO 158687 on 10 March 1977 shows at least three brief occultation events which appear to coincide with portions of a Uranian ring system reported by Elliot *et al.*¹. No occultation by Uranus itself occurred at Perth. Our group also obtained an observation by Smith with a portable telescope from the vicinity of Mauritius. The data are marginal due to variable transparency but it seems clear that there was no occultation by Uranus which diminished the stellar intensity by more than 50%. No ring events were observed from Mauritius due to the onset of a rain shower. Our observations at Perth, along with the null result from Mauritius, can be combined with other available data²⁻⁵ to improve the deduction of the geometry of the ring features, and to obtain a preliminary upper limit to the radius of Uranus.

Our Uranus photometers recorded photon counts at 10 ms intervals on a red and a blue channel simultaneously. The red channel used an RCA C31034 photomultiplier at an effective wavelength of 8,000 Å, while the blue channel accepted light shortward of 5,000 Å, using an uncooled EMI 9789-B tube. The blue channel was used as a monitor of sky transparency and Uranian scintillation and could be used to help reject spurious 'occultation events'. In addition, both channels were chopped to the sky at a nominal frequency of 50 Hz in order to give independent measurements of sky background for point-by-point subtraction. An uncoated pellicle mirror permitted simultaneous guiding. The photon counts were recorded using a portable cassette tape recorder.

Observations at Perth, which enjoyed favourable weather, were made with the 41-cm telescope of the Western Australian University at the Perth Observatory. According to plan, data recording commenced at 20:20 UT, and shortly afterwards an occultation event of almost exactly 1 s duration occurred (Fig. 1). This event was not observed by the adjacent experiment of Millis *et al.* on the 61-cm telescope which was being recentred at the time; possible subsequent low-amplitude events at 20:23:19 and 20:23:43 reported by the latter³ are not apparent above the noise in our data, even at 0.1 s time resolution. At 21:04 UT, our observer switched to a new cassette, during which data recording was interrupted for 20 s. Observations then continued until approaching dawn terminated operations at 21:45 UT. Just before this point, two more 1 s duration

Fig. 1 Initial observation of the α -ring at Perth. Data have been averaged to 1 s time resolution, and the ratio of the signal in the red channel to that in the blue channel is plotted. The stellar base line is approximately at $S_R/S_B = 0.34$. Because of the narrowness of the α feature, at this time resolution the full drop is somewhat washed out. The location of putative events at 20:23:19 and 20:23:43 is indicated.



occultations were observed (Fig. 2). Quite accidentally, because of different observing procedures, none of the three major events observed by Millis *et al.* were also observed by Zellner, or vice versa.

The absolute timing of our data records is established by a time signal placed at the beginning of the first cassette and at the end of the second. Final determination of the times of the occultation events is nearly complete; the preliminary times⁴ already leave little doubt that the three events correspond, in the notation of Elliot *et al.*¹, to a crossing of the α -ring on the immersion side (α_1), a subsequent recrossing of the α -ring on the emersion side (α_2), followed by a crossing of the β -ring (β_2).

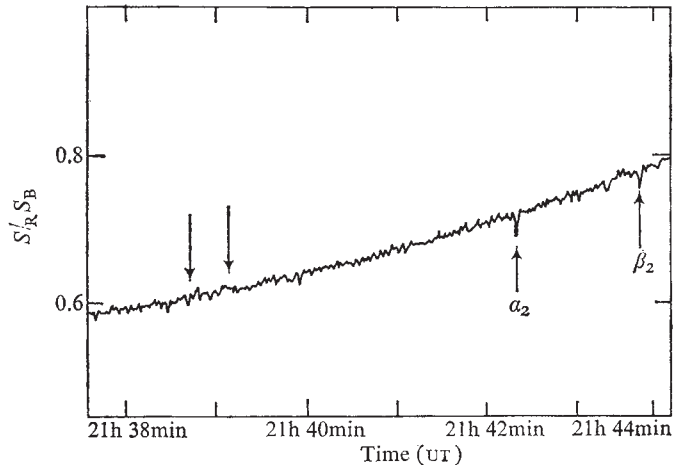


Fig. 2 Subsequent observation of the α -ring and the β -ring on the east side of Uranus. Increasing redness of the sky is caused by the approach of dawn. Possible location of rings interior to α , if they continue around the planet, is also shown.

Inspection of the data at high time resolution shows that the maximum loss of light during the α -occultations is about 50% of the stellar intensity. The β -event is considerably weaker and is only marginally detected against the rapidly brightening sky. We find that the general characteristics of the α -occultations can be reproduced by a model with a randomly-placed array of occulting bodies with dimensions on the order of 1 km or smaller and an areal density $\sim 50\%$ over a band of about 10 km width. For a point source, such an array would produce intense scintillation due to diffraction. Such scintillation is not observed, nor is it expected in view of the angular size of the occulted star. Photometry by Wisniewski indicates that SAO 158687 is probably a K3III star with a projected diameter at Uranus of approximately 4.5 km, and this value has been used in the model.

We have made a preliminary fit to all timings available to us (Fig. 3), under the assumption that the rings are circular features lying in the adopted orbital plane of Uranus' satellites⁶. For each of the five confirmed rings, the coordinates of the centre of the orbit and the radius were adjusted to the best least-squares fit. We have already concluded that neither the inner four rings (α , β , γ , δ) nor the outer ring ϵ are truly concentric circles to within their widths. Consider two concentric circles which are crossed by an arbitrary straight line. Clearly the two line segments contained between the circles are of equal length, and will remain equal if the circles are tilted and projected to form ellipses. The relative times α - β at Perth for immersion and emersion do not satisfy this theorem, nor do many of the other relative times which are available. Small corrections for nonlinearity due to the earth's rotation etc., do not change this conclusion. Although the four inner rings are not quite concentric, and therefore time-variable, the best fits to their centres coincide to within about 100 km, and indicate a further correction⁵ to the Uranus ephemeris (relative to the star) of 0.15 arc s south and 0.015 arc s west. Using the ϵ ring alone, the

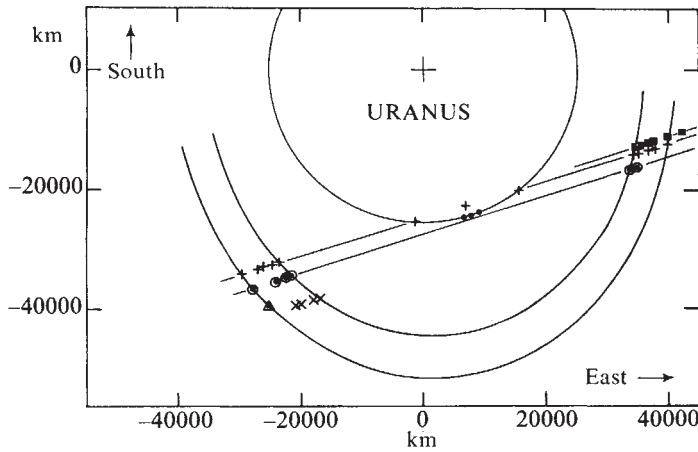


Fig. 3 Uranus occultation events. + observations of Elliot *et al.*¹⁻³; ■ observations of Churms²; ○ observations from Perth^{3,4}; ● observations from Mauritius; △ observations by Bhattacharyya and Kuppaswamy^{3,4}; x observations by Mahra and Gupta.

indicated southward correction is 0.05 arc s and the westward correction is 0.015 arc s.

The degree of consistency between the ring centres can be used to locate the centre of mass of Uranus to within the same accuracy. This assumption is only valid to the extent that the limited sample of the rings from the occultation chords is not systematically affected by local ring distortions. If this is so, we then conclude that the null result at Mauritius together with the inner ring solutions places an upper limit to the radius of Uranus of 26,300 km, in opposition to a value of $27,900 \pm 500$ km proposed earlier by Smith⁷. A radius as small as 25,100 km would be consistent with the ϵ -ring solution. We may be able to determine the radius more accurately by using the atmospheric occultations (Elliot *et al.*¹ and Churms²), if there is also an occultation of a few per cent in the stellar intensity at Mauritius. This effect will be sought if we can successfully correct for the effects of clouds to this accuracy.

Our group has also attempted observations from the 91-cm telescope at Hartbeespoort, South Africa, and from the 102-cm telescope at Sutherland, South Africa. We also participated in a joint experiment with the University of Cape Town on the Sutherland 188-cm telescope. None of these experiments obtained data because of heavy cloud cover at both sites.

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Wave optics of the central spot in planetary occultations

THE detection of a bright central spot¹ during the occultation of ϵ Geminorum by Mars² has demonstrated that an exponential planetary atmosphere can act as a lens with somewhat peculiar properties. In the course of a general study of certain wave-optical phenomena in planetary occultations, we have carried out an investigation into the diffraction nature of the central spot, which has not been previously considered in occultation theory. Some of the results are presented in this letter, along with implications for possible future experiments.

Assume that an observer is at a distance D from a spherical planet of radius R . The planet has a refracting, non-absorbing, exponentially stratified atmosphere with scale height H . Observations are made at wavelength λ . We assume that $(\lambda D)^{1/2} \ll H$, $\lambda \ll (HR)^{1/2} \ll D$, and $R^{3/2} \ll DH^{1/2}$. Then the planet can be regarded as a phase object³ which modifies the phase of an incident plane wave from a distant point source by

$$\Phi_A = \alpha \lambda^{-1} \exp(-r_1/H) \quad (1)$$

where α is a constant and r_1 is the distance of closest approach of the optical path to the centre of the planet. The optical axis is then the straight line linking the centre of the planet and the distant source. Let the distance of the observer from this axis be r . Superimposing the partial waves, we calculate the intensity for $r \ll R$

$$I(r) = (2\pi)^2 \frac{RH}{D\lambda} J_0^2 \left(\frac{2\pi Rr}{D\lambda} \right) \quad (2)$$

normalised such that the unocculted intensity $I(\infty) = 1$. The distribution of intensity resembles the Airy function and has a typical width

$$w \sim D\lambda/R \quad (3)$$

For $w \ll r \ll R$, the average intensity goes over to the previously-derived^{1,4} ray-optical result, $I \sim 2H/r$.

These results imply that, in principle, the central occultation spot could be observed to obtain very high resolution of distant objects. Assume $H = 10$ km, $D = 1$ AU, $\lambda = 5,000$ Å, and $R = 4,000$ km (Mars or Venus). The width of the central spot image of a point source is then $w \sim 2$ cm, corresponding to a theoretical resolving power of $\sim 10^{-13}$ radians (equivalent to 4 km at 1 pc). The light gathering power of the 'telescope' can be expressed in terms of an equivalent aperture R_e for a conventional telescope:

$$R_e^2 \sim I(0) w^2 \quad (4)$$

We find $R_e \simeq 100$ m for this example.

Since central occultations by suitable planets are rarely seen from the Earth's surface, presumably use of the central spot technique would require the use of spacecraft to place the observer at the image of the source to be investigated. In principle, one could profitably use the technique to observe such objects as quasars, white dwarfs, and pulsars. However, certain grave practical difficulties must be mentioned:

(1) The point spread function of equation (2) is not as well-defined as the Airy function. The sharp central peak has broad wings which fall off as r^{-1} . Deconvolution of images of extended sources would thus be difficult, although objects such as binary point sources could probably be readily identified. (2) It would evidently be quite difficult to locate the image of a true point source because of its small size. (3) A real planet would be an imperfect lens. The optical axis is poorly defined for an oblate

planet, such as Mars¹. This problem could be reduced by using a slowly rotating planet, such as Venus. Since the atmosphere will in general be turbulent, the instantaneous image may differ considerably from the ideal image shown by equation (2).

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Video tape recording of a possible ball lightning event

PHOTOGRAPHS purporting to be of ball lightning have been published by a number of authors, as discussed for example by S. Singer¹. More recently, what is believed to be the first photograph of the formation of ball lightning has also been presented^{2,3}.

For the past four years I have been associated with a lightning research programme in the Transvaal Highveld region of southern Africa, in the course of which various photographic techniques have been applied routinely to the study of several aspects of the lightning discharge. Incorporated among these techniques has been the use of an automatic video tape recording system using a television camera with a silicon diode array target tube, in a manner similar to that originally reported by Winn *et al.*⁴. Particular advantages of this system in comparison with conventional photography include an enhanced sensitivity and sufficient time resolution to resolve the majority of multiple-stroke flashes. Over the past two lightning seasons some 500 lightning strokes to ground have been recorded and studied using this system.

During a thunderstorm on the evening of the 12 November 1976, a video tape recording which included a ground flash exhibiting particularly unusual characteristics was obtained. The resultant video tape was played back field by field and the individual still pictures rephotographed on a conventional display monitor. The complete event, which lasted for approximately 180 ms, is shown in Fig. 1. (The nominal time resolution of the video recording system is the

time taken to sweep one field, that is, 20 ms—hence the intervals indicated in Fig. 1.) Inherent in the operation of the silicon target array is the ability to retain images of very short duration luminous events. Therefore, each still photograph constitutes an 'integrated' record—sampled at 20 ms intervals—of what is in all probability a complex process, exhibiting several stages of transient re-illumination.

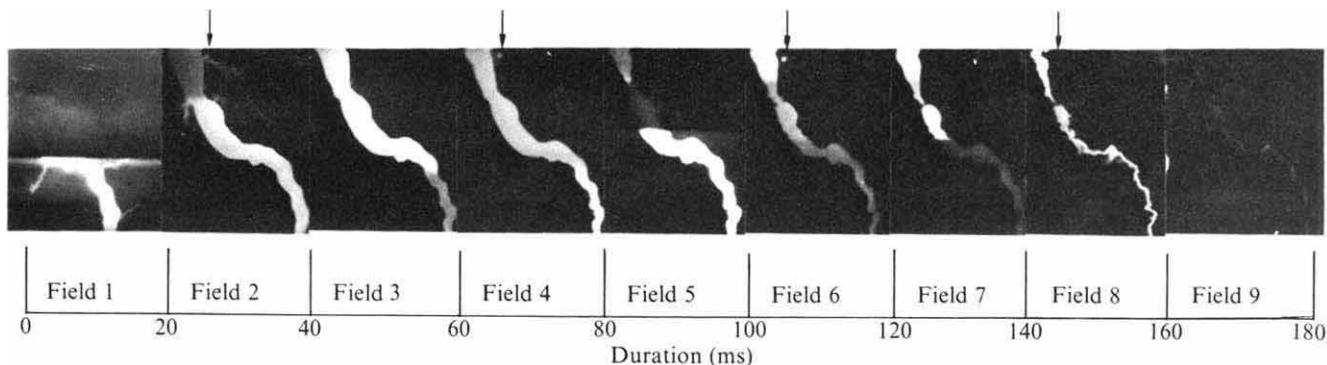
The first field in the sequence shows a faint image of a downward leader, together with the subsequent bright return stroke—which must have occurred at a time when about half the field had already been swept during the raster scan. (The 'blooming' of the very bright return stroke image is a feature of this type of camera—particularly for intense nearby strokes such as this flash, which occurred about 700 m from the camera—as determined from time-to-thunder measurements).

Thereafter, in the remaining fields of the sequence, a further three subsequent return strokes may tentatively be identified. (The main criterion for the identification of a return stroke is for a significant enhancement of channel luminosity to be apparent in relation to the images on preceding fields. Although the silicon diode camera has inherently low image persistence, remanent image persistence can occasionally make the identification of subsequent strokes rather difficult, especially in the event of short inter-stroke intervals).

Of particular interest in this sequence, however, is the recurrent appearance of a ball-shaped image in fields 2, 4, 6 and 8, apparently detached and to the right of the top portion of the main channel, except in field 2 where its position seems to coincide with that of a branch. It is interesting to observe (in Fig. 1) that the 'ball' re-illuminations seem to have occurred during the inter-stroke intervals, rather than at the times when the main discharge channel was re-illuminated by the passage of the bright return strokes. This would tend to discount the possibility of the ball being an artefact—arising as the consequence of a blemish on the lens, for example, or from a raindrop on the camera face. (No such blemish was recorded elsewhere on the video tape and no rain had occurred at the time when this flash was noted, nor for more than 10 min after the flash).

The final return stroke is followed by an indication of the persistence of a decreasing 'continuing current', as is known occasionally to occur⁵ and as has frequently been recorded in the course of my measurements. However, the images of the channel in fields 8 and 9 are unusual in that they display an excessive tortuosity, together with what may be construed as 'beading'. The latter phenomenon is frequently reported to be associated with ball lightning events¹

Fig. 1 Video-taped image sequence of multiple-stroke lightning flash showing possible appearance of ball lightning. Field 1, leader and first return stroke. Field 2, fading image of first return stroke; first appearance of ball-shaped image. Field 3, second return stroke. Field 4, fading image of second return stroke; second appearance of ball-shaped image. Field 5, third return stroke (during scan). Field 6, fading image of third return stroke; third appearance of ball-shaped image. Field 7, fourth return stroke (during scan). Field 8, persistence of continuing current ('beading'); fourth appearance of ball-shaped image. Field 9, fading of final image.



and, as has been suggested, may be produced in an analogous mechanism⁶. Some instances of excessive tortuosity and related re-illuminated beaded processes have also been observed recently in the French research programme⁷.

If one assumes that the ball image recorded here is associated with the lightning discharge, then, from a knowledge of the flash distance, the image height may be determined and is found to be approximately 300 m above ground while the apparent image diameter is about 5 m. The latter dimension, although rather large in comparison to many reputed observations of ball lightning, is not inconsistent with recent reports^{2,3}, but may also be the consequence of the enhanced sensitivity of the camera and the tendency towards image 'blooming'.

It should also be noted that this particular flash struck the ground about 300 m from a 60 m high aluminium mast. This implies that at least one of the conditions was met for one class of theoretical mechanisms for the formation of ball lightning, that is, those requiring the production of intense clouds of positive space charge in the vicinity of the downward progressing negative leader for example⁸, or those requiring field enhanced emission during upward interconnecting positive leader processes from grounded conductive prominences in close proximity to the descending negative leader⁹.

In conclusion, however, it should be emphasised that this record is presented mainly for comment and possible interpretation. Clearly, positive identification of this event as a ball lightning event must be tentative until corroborated by further evidence. Meanwhile this observation may improve understanding of the relationship between the lightning discharge and the processes leading to the formation of ball and beaded lightning.

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Open system crystal fractionation and incompatible element variation in basalts

O'HARA¹ has recently proposed an open-system model for the fractional crystallisation of magmas involving continual replenishment of the magma chamber and only partial extraction of the fractionated liquid at each stage. This could produce a series of chemically uniform lavas (such as basalts) with very little resemblance to the primary mantle melt. It is claimed that the model can also explain large variations in the concentrations of incompatible trace elements and their inter-element ratios which would otherwise be ascribed to variable degrees of partial melting or inhomogeneity in the mantle source region. The purpose of this note is to explore the potential and limitations of O'Hara's model in this respect and to test it against the observed variability of Icelandic basalts.

I suggest that three rare earth elements (Ce, Sm and Yb) are especially useful for this purpose. In most solid-liquid

equilibria relevant to basalt genesis they range from incompatible to slightly compatible with increasing atomic number. Their relative crystal-liquid partition coefficients are better understood than for many other trace elements although there is some discrepancy between experimentally determined values and estimates based on phenocryst-matrix relationships (Table 1). Lastly there is a large amount of accurate information on the concentrations of these elements in basalts, determined by mass-spectrometric isotope dilution.

The theoretical behaviour of incompatible elements during magmatic processes is reviewed by Hanson² to whom the reader is referred for general discussions and relevant equations. Here I have attempted to compute geologically realistic models for the significant factors in basalt magma evolution and the results are plotted in Figs 1 and 2 for Ce against Ce/Sm and Ce/Yb enrichment, relative to concentrations in the source. Brief notes on the details of each calculation follow.

● **Partial Melting.** An initial source composition of 55% olivine, 25% orthopyroxene, 15% clinopyroxene and 5% garnet was assumed, with these minerals entering the melt in the following relative proportions (melting modes): 10%, 20%, 40% and 30% respectively. After the disappearance of any phase (for example, garnet at 15% partial melting) the melting modes for the remaining minerals were recalculated to 100% in the same relative proportions as before. Partition coefficients used were set 1 in Table 1, since it was felt that these experimentally determined values were more appropriate to equilibrium conditions pertaining during mantle melting. Two curves are shown in the figures—*a* represents batch melting in which the melt remains in equilibrium with the residual solids, *b* is for incremental melting in which each successive 1% of the melt is removed as soon as it forms.

● **Zone refining.** In this process³ melt advances by simultaneously assimilating material above it and depositing cumulates behind it, the main variable being the number of relative zone lengths processed, that is, the number of times the magma consumes its own volume. In as far as this process can be visualised at all, it must be restricted to high temperature regions of the mantle through which the basalt magma rises. Consequently the magma was assumed to be a 30% partial melt as in curve *a* above and the bulk partition coefficients for zone refining were calculated as equivalent to 60% olivine, 25% orthopyroxene and 15% clinopyroxene, using set 1 from Table 1. The results are plotted as curve *c*.

● **Closed-system crystal fractionation.** The parent magma was again assumed to be the 30% partial melt defined above, evolving by crystal fractionation according to the Rayleigh law. To allow for changes which would occur in major element chemistry during this process, it was assumed that the first 30% of solids formed would correspond to peridotite (50% olivine, 30% orthopyroxene and 20% clinopyroxene), the next 40% to olivine-gabbro (30% olivine, 30% clinopyroxene and 40% plagioclase) and the final 30% to anorthosite or granite (30% clinopyroxene, 70% plagioclase). The addition of quartz to the final stage has little effect on relative fractionation of these elements. For this supposed high-level process, the partition coefficients of set 2, based on phenocryst-matrix values, were preferred.

● **Open-system crystal fractionation.** O'Hara's basic equation (*ibid.* Fig 3) for the enrichment of a trace element in the steady state liquid contains three variables: *D*, the bulk partition coefficient, *X*, the proportion of the magma within the chamber which crystallises as cumulates and *Y*, the proportion of the fractionated magma which is removed and erupted as a lava. The relative fractionation of two elements with similar (low) *D* values depends principally on the ratio *X/Y*, reaching effective saturation for Ce/Sm at about 10⁴

Table 1 Crystal-liquid partition coefficients for basaltic melts

		Olivine	Orthopyroxene	Diopside	Augite	Garnet	Plagioclase
Set 1*	Ce	0.0010	0.003	0.098	—	0.021	—
	Sm	0.0013	0.010	0.26	—	0.22	—
	Yb	0.0015	0.049	0.28	—	4.00	—
Set 2†	Ce	0.0069	0.024	—	0.15	—	0.12
	Sm	0.0066	0.054	—	0.50	—	0.067
	Yb	0.014	0.34	—	0.62	—	0.067

*Data from experimental studies and clinopyroxene-mineral comparisons, after Hanson².

†Data from phenocryst-matrix studies of basaltic rocks, after Arth and Hanson⁴.

and for Ce/Yb at about 10^3 . At the same time, the maximum enrichment of any of these elements increases with X , but is essentially constant below $X = 0.1$. The lines plotted in the figures were thus calculated for $X = 0.01$ by varying X/Y as marked, with the effect of increasing X indicated by the dotted lines. Two cases were considered: with bulk partition coefficients corresponding to each of the first two stages in the closed-system model (that is, peridotitic and olivine-gabbro cumulates) respectively. In both a 30% partial melt was again taken as the primary magma, but for the olivine-gabbro fractionation case it was further assumed that this underwent 30% crystallisation of peridotitic cumulates before arrival at the high-level magma chamber, resulting in a fivefold previous enrichment of Ce, Sm and Yb relative to original source concentration.

The general features of Figs 1 and 2 are essentially similar and are not critically dependent on the mineralogical details of the models. The effect of the choice of partition coefficients is more significant, but it should be noted that the use of set 2 for the high-level crystal fractionation processes enhances their ability to discriminate between Ce and the other two elements. Nevertheless, partial melting is still by far the most efficient mechanism for fractionating Ce/Sm and Ce/Yb ratios, increasing these by factors of about three and 10 respectively for only a 50-fold enrichment of Ce relative to the source. Furthermore, incremental or repeated partial melting is the only process considered capable of producing a magma with lower Ce/Sm and Ce/Yb ratios than the original source material (although repeated zone refining could theoretically have

a similar effect). At the other extreme closed-system crystal fractionation is scarcely at all effective at fractionating these elements with respect to each other—even less so than is normally considered for models with constant D_s . This is because, in the third stage modelled here, removal of heavy rare earth elements in clinopyroxene is largely compensated by the preference of plagioclase for Ce. Zone refining also seems to have a negligible effect except at

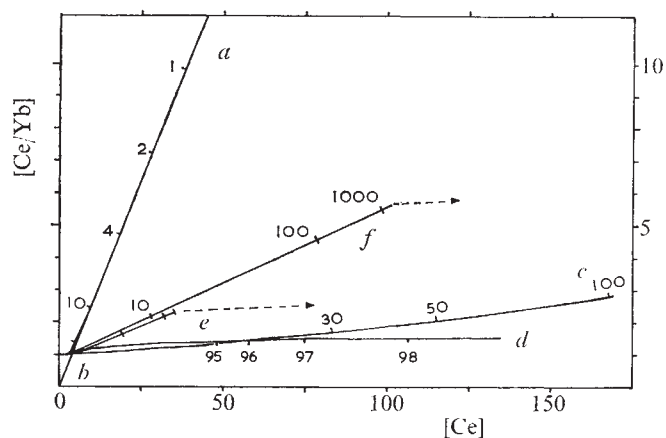


Fig. 2 Ce enrichment against increase in Ce/Yb ratio; see Fig. 1 legend for explanation.

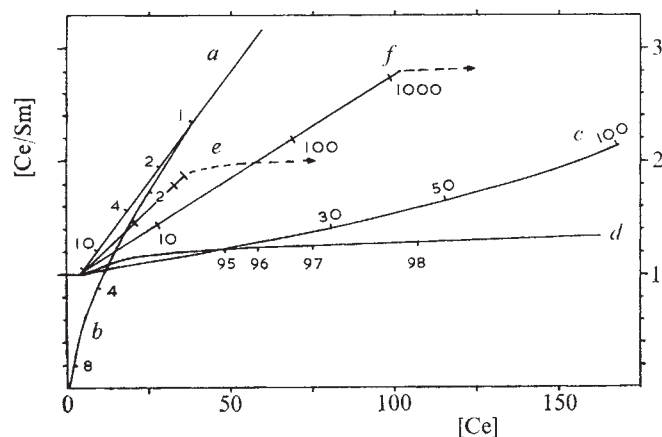


Fig. 1 Ce enrichment against increase in Ce/Sm ratio (both relative to a value of one for the mantle source) for various magmatic processes (see text for details). *a*, Equilibrium batch melting, % melt marked. *b*, Incremental melting (1% increments), % melt marked. *c*, Zone refining in the mantle, no. of relative zone lengths processed marked. *d*, Closed-system crystal fractionation, % crystallisation marked. *e*, Open-system crystal fractionation of olivine-gabbro cumulates according to O'Hara's¹ equation without assimilation, $X = 0.01$, X/Y marked; dotted portion shows effect of increasing X for $X/Y = 10^4$. *f*, As for *e* but with peridotitic cumulates.

improbably high values for the number of zone lengths processed (>100). The curves plotted for open-system fractionation are intermediate in their efficiency at fractionating Ce/Sm and Ce/Yb ratios, but the case involving olivine-gabbro cumulates has quite low limiting values (about two times for Ce/Sm and 2.5 times for Ce/Yb).

The Icelandic basalt data against which the models are to be tested are summarised in Table 2 under four separate headings, each of which will be considered in turn. In making an initial assessment it is not necessary to know the actual abundance of Ce in the mantle source, although the reader may find it interesting to bear in mind that this is commonly estimated at somewhere between one and three times chondritic abundance.

The first grouping includes all basalts with a common $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.70315 (ref. 7), which would thus permit a common source region or parent magma. These are the majority of postglacial mainland basalts and the alkali-olivine basalts of Surtsey and Heimaey. They show a modest increase in Ce/Sm and about a threefold increase in Ce/Yb for an overall sixfold increase in Ce abundance. It is clear that the closed-system crystal fractionation model cannot explain these variations and that zone refining fails for the Ce/Yb variation (except at vanishingly low source abundances and reprocessing of several hundreds of magma volumes). At face value either partial melting or open-system crystal fractionation could be responsible. For example, batch melting of from 5% to 30% of a common source would work with original abundances about six

Table 2 Ce, Sm and Yb variations in Iceland

Grouping	Ranges*		
	Ce	Ce/Sm	Ce/Yb
I Post-glacial tholeiites and alkali basalts with constant Sr/Sr	16-93	1.0-1.6	1.2-3.8
II Reykjanes Ridge tholeiites	7-16	0.5-1.0	0.5-1.2
III All basalts	2-93	0.4-2.3	0.3-7.1
IV All volcanics	16-210	1.0-2.9	1.2-7.4

*All normalised to chondritic abundances.
Data from refs 5-7.

times chondrite. The implied source abundance would reduce to acceptable values if it were allowed that the basalts underwent some crystal fractionation of olivine on their way to the surface (as O'Hara¹ shows must be the case from consideration of their Fe/Mg ratios). Open-system fractionation as modelled here would require initial source abundances lower than those for simple partial melting by a factor of two or more, apparently a point in its favour. However, the olivine-gabbro case can easily be eliminated: in order to discriminate effectively between Ce and Sm, this process would have an even more marked discrimination between trivalent and divalent Eu as a result of the removal of plagioclase. Calculations parallel to those illustrated here show that negative Eu anomalies of at least 30% would be produced, whereas none are actually observed in any Icelandic basalt. This leaves the possibility of open-system fractionation of peridotite cumulates. In assessing whether this is a geologically feasible alternative to the previously proposed idea that these rocks result from variable degrees of partial melting of a common source^{5,6}, the following factors require re-emphasising. (1) The selection of partition coefficients for these models favours the open-system crystal fractionation case, perhaps unduly. (2) The proportion of magma withdrawn from the magma chamber (Y) must be exceedingly low—certainly 0.1% or less. (3) Failure to achieve the steady state postulated by O'Hara would also reduce the amount of inter-element fractionation. (4) Finally, since the absence of plagioclase fractionation restricts operation of the process to the early stages of evolution of the parent magma, possibly at considerable depths, the distinction between this and other mantle processes such as differential partial melting may be somewhat academic.

The second group shows the incompatible element variation along the Reykjanes Ridge south of Iceland (see also refs 8 and 9). Here a twofold increase in Ce/Sm (slightly more for Ce/Yb) is accompanied by little more than a twofold increase in Ce. Even equilibrium partial melting from a common source cannot account for such a rapid increase, although incremental partial melting could. When the sympathetic variation of ⁸⁷Sr/⁸⁶Sr and ²⁰⁷Pb/²⁰⁴Pb ratios is taken into consideration⁹⁻¹¹ the only reasonable explanation is one which invokes inhomogeneity in the mantle source regions, although this could well have resulted from previous extraction of partial melts, which would also explain the low chondrite-normalised Ce/Sm and Ce/Yb ratios.

Comparison with Figs 1 and 2 demonstrates that group III (all Icelandic basalts) and IV (all post-glacial volcanics, including intermediate and acid ones) must be interpreted similarly. Indeed, there is no process realistically capable of causing such high enrichments in Ce as would be implied by the derivation of Icelandic rhyolites from a mantle source which would also yield the light rare earth element-depleted basalts. The conclusion of O'Nions and Gronvold⁹ that the acid rocks represent partial melts of a pre-enriched (that is, crustal) source seems well borne out by their significant negative Eu-anomalies.

O'Hara's¹ second line of argument was that if the magma chamber advanced into the oceanic crust, assimilation of basalt which had previously interacted with seawater would very much accentuate enrichment of incompatible elements and could lead to domination of the trace element and isotope chemistry by the characteristics of seawater. This could indeed explain some of the variation in ⁸⁷Sr/⁸⁶Sr ratios in, for example, island arcs. It is totally inapplicable to the case of Iceland for the following reasons. Firstly, there is no indication that seawater interaction has ever affected basalts either on the Icelandic mainland or along the Reykjanes Ridge—the observed variations in ⁸⁷Sr/⁸⁶Sr being fully confirmed by the evidence of Nd isotopes¹². In submarine basalts from DSRP Leg 37 which do show evidence of Sr-isotope contamination¹³ the Nd isotopes are unaffected¹². Second, rare earth concentrations are little affected by seawater alteration—the contaminated Leg 37 basalts still have flat-to-light-depleted rare earth element patterns¹³ and assimilation of such rocks could not account for enrichment in Ce/Sm or Ce/Yb ratios as displayed by alkali basalts from Iceland or elsewhere.

In conclusion, open-system crystal fractionation as proposed by O'Hara¹ may be a more geologically reasonable model for the modification of mantle-derived magmas than the conventional closed-system model and seems capable of producing greater variability in incompatible trace element ratios. In comparison with partial melting, however, it is severely limited in application to one of the best studied volcanic regions and cannot account for more than a small portion of the observed variation in Icelandic volcanics. In particular, it is not a viable alternative in those cases where mantle inhomogeneity has been previously invoked on the basis of trace element data. The true nature and causes of mantle inhomogeneity beneath Iceland and the Reykjanes Ridge have long been problematical^{7-11,14} but, as for mid-ocean ridge segments in general, there is no escaping the need for the redistribution of incompatible trace elements, probably involving a partial melting process at some stage.

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Palaeoatmospheric argon in Rhynie chert

THE isotopic compositions and relative abundances of rare gases in the Earth's atmosphere impose rigid constraints on models of atmospheric evolution. Appreciable quantities of argon from the Devonian atmosphere have been extracted from silicified fossil plants and the isotopic composition of this argon implies that the Earth must have been strongly outgassed early in its history.

The fundamentally different origins of ⁴⁰Ar and ³⁶Ar in the Earth's atmosphere (radiogenic and primordial respectively) make the atmospheric (⁴⁰Ar/³⁶Ar) ratio a sensitive

parameter for investigating the evolution of the Earth's interior. The ($^{40}\text{Ar}/^{36}\text{Ar}$) ratio in the mantle is also potentially valuable, but atmospheric contamination and the inhomogeneous distribution of potassium within the Earth make the characterisation of this component equivocal, and seriously weaken the argon degassing models which have been based on it^{1,2}. The argon in the Earth's atmosphere, unlike that in the mantle, is always well mixed isotopically and represents the time-integrated product of chemical fractionation, radioactive decay and degassing processes within the Earth.

All of the ^{36}Ar in the atmosphere was originally trapped within the Earth when it was first formed. A theoretical value of 2×10^{-4} has been calculated for the ($^{40}\text{Ar}/^{36}\text{Ar}$) ratio of this primordial argon component³ and an experimentally determined upper limit of $(1.4 \pm 0.6) \times 10^{-3}$ has been deduced from the analyses of meteorites⁴. Almost all of the ^{40}Ar in the atmosphere ($6.54 \times 10^{19} \text{ g}$) is therefore the result of the decay of naturally occurring ^{40}K , the long half life of which must have effectively decoupled the entry of the two isotopes into the atmosphere. If it is assumed that ^{36}Ar was concentrated in the crust/atmosphere more readily than potassium, then the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio in the atmosphere must have increased monotonically from an initial value of less than unity to the present day ratio of 295.5, the precise shape of the evolution curve depending on the variation of the argon degassing rate constants with time^{5,6}. The experimental determination of the shape of this evolution curve should therefore allow argon degassing processes to be critically investigated⁷.

The isolation of palaeoatmospheric argon requires the existence of geological samples which (1) are authigenic, (2) incorporated atmospheric argon during their formation, (3) are low in potassium, (4) are very old, (5) are very retentive, and (6) have had a simple geological history. Cherts are among the most suitable samples, but previous attempts to detect palaeoatmospheric argon in Precambrian cherts proved unsuccessful, possibly as a result of their marine origin and complex geological history⁷. The samples selected for analysis in this work were chips of Rhynie chert from the Lower Devonian (about 380 Myr) of Aberdeenshire, Scotland. The Rhynie chert is very low in potassium (100–150 p.p.m.), contains abundant silicified fossils of some of the oldest known land plants and is extremely well preserved⁸.

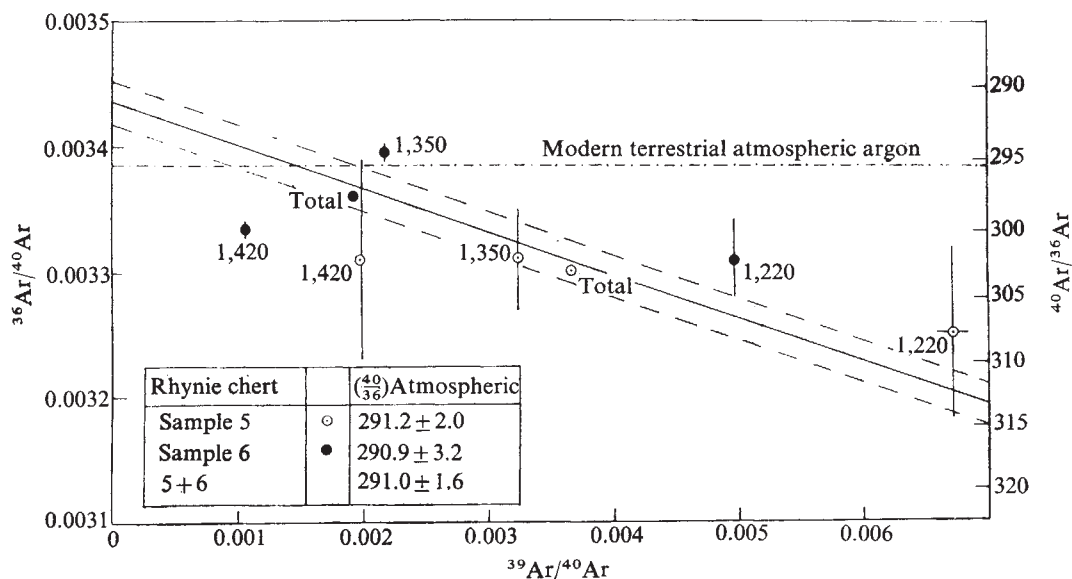
The samples, weighing 50–200 mg, were analysed by the $^{39}\text{Ar}/^{40}\text{Ar}$ neutron activation method¹³ so that corrections for the *in situ* decay of ^{40}K could be applied. No

corrections for the neutron-induced production of ^{36}Ar and ^{39}Ar from calcium could be made, based on the ^{37}Ar , because of the long elapse time between irradiation and analysis, but the total amounts of trapped ^{36}Ar were remarkably high ($2\text{--}3 \times 10^{-8} \text{ ml STP g}^{-1}$) and the measured calcium content was so low (0.012%) that it could only have reduced the total ($^{40}\text{Ar}/^{36}\text{Ar}$) ratios by 0.01%, or 0.03. The total ($^{40}\text{Ar}/^{36}\text{Ar}$) ratios from two unirradiated samples (297.9 ± 1.5 and 295.7 ± 1.1) were in fact less than from the irradiated samples (303.1 ± 3.2 and 299.2 ± 0.8). The ^{36}Ar thermal release patterns from the irradiated samples (5 and 6) were bimodal, most of the ^{36}Ar being released above 1,200 °C, with maxima at 1,350 and 1,420 °C respectively. These very high extraction temperatures strongly suggest that at least the high temperature ^{36}Ar component must be occluded palaeoatmospheric argon. The release patterns for the potassium derived ^{36}Ar were broader, with maxima at slightly lower temperatures (1,220 and 1,350 °C respectively)⁹.

The use of the $^{39}\text{Ar}/^{40}\text{Ar}$ method enables corrections for the ^{40}Ar produced by *in situ* decay of ^{40}K to be applied, so that the isotopic composition of the trapped component can be determined. On a correlation plot of $^{36}\text{Ar}/^{40}\text{Ar}$ against $^{39}\text{Ar}/^{40}\text{Ar}$, mixtures of trapped and potassium-derived argon define a mixing line between pure trapped argon ($^{39}\text{Ar}/^{40}\text{Ar} = 0$) and pure potassium-derived argon ($^{39}\text{Ar}/^{40}\text{Ar} = 0$)¹³. The ($^{39}\text{Ar}/^{40}\text{Ar}$) ratios were so low that the ($^{40}\text{Ar}/^{36}\text{Ar}$) ratios from all gas fractions were best corrected individually for ^{40}Ar produced *in situ*, assuming an age of 380 Myr. ($J = 0.0218$, ($^{39}\text{Ar}/^{40}\text{Ar})_{\text{K}} = 0.098$). The weighted mean of these corrected ratios for all gas fractions from samples 5 and 6 were (291.0 ± 1.3) and (290.9 ± 1.5) respectively, with an overall weighted mean of (291.0 ± 1.0). The weighted mean atmospheric ratio for all of the low temperature (<1,000 °C) gas fractions (291.7 ± 0.7) was indistinguishable from the total high temperature weighted mean (291.0 ± 1.6), implying that the low temperature atmospheric component may also be ancient, rather than recently absorbed, atmospheric argon.

The high temperature gas fractions are plotted on a three-isotope correlation diagram in Fig. 1. It can be seen that the 1,350 and 1,420 °C extractions from sample 6 do not fall on the mixing line within their analytical errors. Some of this discrepancy may result from calcium interference, but it should be noted that for both samples, the corrected ($^{40}\text{Ar}/^{36}\text{Ar}$) ratios increase with extraction temperature: 1,220 < 1,350 < 1,420 °C. This would be expected if argon extraction in the laboratory involves

Fig. 1 Isotope correlation plot of ($^{36}\text{Ar}/^{40}\text{Ar}$) against ($^{39}\text{Ar}/^{40}\text{Ar}$) for the high temperature ($T > 1,000$ °C) extractions from two samples of neutron-irradiated Rhynie chert (temperatures in °C). The calculated weighted mean corrected atmospheric ($^{40}\text{Ar}/^{36}\text{Ar}$) ratio is 291.0 ± 1.6 assuming a gas retention age of 380 Myr. The points marked 'total' represent the arithmetic sums of the high temperature points from each sample and they both fall slightly below the mixing line because the points have not been individually weighted according to their analytical errors.



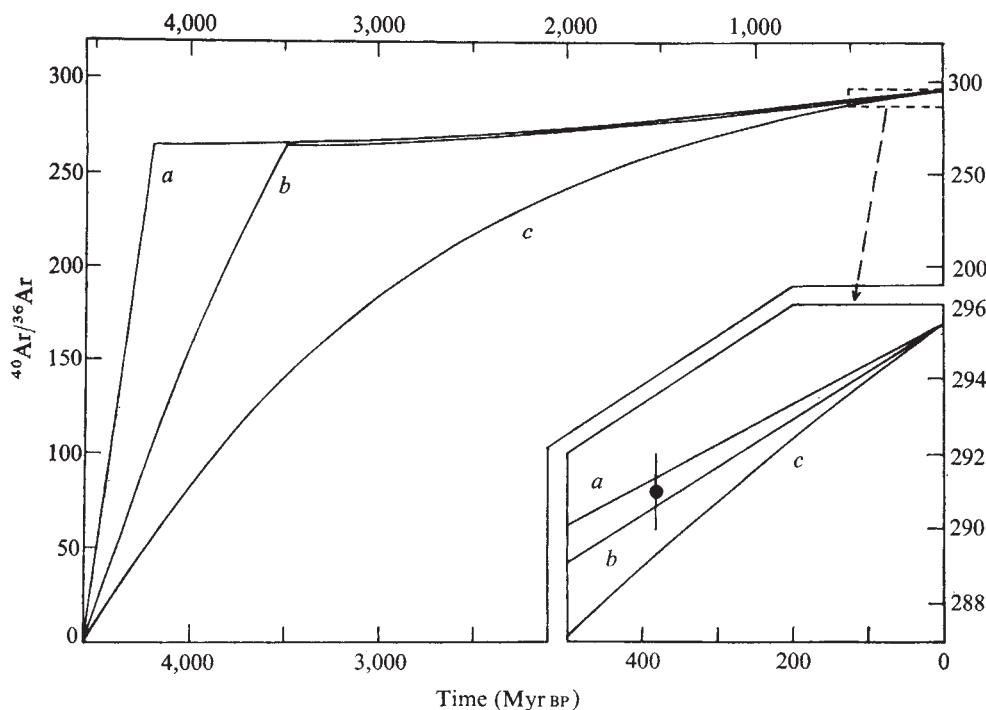


Fig. 2 Evolution curves for the ($^{40}\text{Ar}/^{36}\text{Ar}$) ratio in the Earth's atmosphere assuming total loss of ^{36}Ar to the atmosphere 4.55×10^9 yr ago. *a*, *b*, Two stage degassing models delimiting a range in potassium content for the Earth from 350–140 p.p.m. and catastrophic degassing periods before 4.2×10^9 and 3.5×10^9 yr ago respectively. *c*, Upper limiting case for single stage degassing models, corresponding to $\alpha \gg \lambda_{40}$ and a potassium content for the Earth of only 75 p.p.m.

mass-dependent diffusion. In these conditions the lighter isotope, ^{36}Ar , may be released more readily than the heavier isotope, ^{40}Ar , giving rise to artificially low initial ($^{40}\text{Ar}/^{36}\text{Ar}$) ratios. The concordance of the palaeoatmospheric ($^{40}\text{Ar}/^{36}\text{Ar}$) ratios from the two samples argues against geological diffusive fractionation and both effects could be critically investigated by measuring ($^{38}\text{Ar}/^{36}\text{Ar}$) ratios in larger, un-irradiated samples. But even without the determination of the isotopic ratio of the trapped component, the fact that large amounts of ^{36}Ar are so strongly bound within the sample is in itself a very significant finding.

An atmospheric ($^{40}\text{Ar}/^{36}\text{Ar}$) ratio of (291.0 ± 1.0) 380 Myr ago imposes a firm constraint on argon degassing models of the Earth. This value is higher than those predicted by all first order degassing models^{5,7}, unless it is postulated that ^{36}Ar is retained in the mantle preferentially to potassium. The degassing rate constant necessary to account for all of the ^{40}Ar in the present day atmosphere depends on the potassium content of the Earth. The most extreme first order model, for example, predicts a ($^{40}\text{Ar}/^{36}\text{Ar}$) ratio as high as 289.0, 380 Myr ago but requires a potassium content for the Earth of only 75 p.p.m. (Fig. 2). To explain the slightly higher measured ratio, the degassing constant must have decreased as a function of time. The simplest model consists of two stages, during the first of which the degassing constant was large compared with the decay constant of ^{40}K , as in the extreme single-stage first-order model. Since the termination of this continuous degassing period, the degassing constant has been small. Other simple models might invoke a degassing constant which has decreased linearly, or exponentially, with time. There exists a family of possible two-stage evolution curves for the atmospheric ($^{40}\text{Ar}/^{36}\text{Ar}$) ratio, depending on what value is taken for the potassium content of the Earth (Fig. 2). This is not known precisely but is constrained by the measured global heat flow. It could be as high as the chondritic value of 880 p.p.m., but the uranium-to-potassium ratios in a wide range of terrestrial rocks are remarkably constant (about 10^{-4}) and significantly higher than in chondritic meteorites¹⁰, implying a potassium content of between 250 p.p.m. and 165 p.p.m., or even less for an initially hot Earth¹¹. This range in potassium content corresponds to a continuous degassing period which terminated between 4,000

and 3,700 Myr ago. The uncertainty in this time which arises from the error in the measured atmospheric ratio ($< \pm 40$ Myr) is minor compared with that arising from the uncertainty in the potassium content of the Earth and from the limitations inherent in the simplified model. The two-stage evolution curves closely follow one another during the second stage and therefore the determination of the palaeoatmospheric ($^{40}\text{Ar}/^{36}\text{Ar}$) ratio in suitable Precambrian samples would provide a crucial test for the model.

The determination of the ($^{40}\text{Ar}/^{36}\text{Ar}$) ratios in the Devonian atmosphere provides further evidence for the early catastrophic degassing of the Earth¹⁴. Thermal history calculations¹¹ and lead isotope studies¹² imply early chemical differentiation and crust formation. This crust must then have remained open with respect to argon for about 700 Myr after the formation of the Earth, a process which may have been facilitated by the same intense meteoritic bombardment to which the moon was subjected, a bombardment which terminated about 3,850 Myr ago. The continuous degassing period suggested by this work is certainly consistent with the absence of terrestrial rocks with known crystallisation ages much in excess of 3,750 Myr (ref. 15).

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Lead isotope measurements from the oldest recognised Lewisian gneisses of north-west Scotland

No isotope ages exceeding ~2,800 Myr have yet been reported from Lewisian gneisses. Furthermore, there is no published isotopic evidence suggesting that Lewisian gneisses incorporate a significant proportion of much older, re-worked sialic crust. Recently, Davies¹ has interpreted field evidence from small, discordant amphibolite bodies as indicating a pre-2,800-Myr history of the Lewisian Complex in the type localities of Scourie and Laxford, Sutherland, which is directly comparable with that of the Godthaab area of West Greenland. He has proposed that equivalents of the ~3,700-Myr-old Amitsoq gneisses and ~2,800-Myr-old Nûk gneisses can be distinguished, on the tentative assumption that the basic bodies are the equivalents of the Ameralik dykes²⁻⁴. This paper tests this hypothesis using Pb isotopes.

In Table 1, we present 12 whole rock Pb isotope analyses on the Older ('Uamhaig') gneisses of Davies¹, as well as eight analyses on other nearby granulite gneisses within the type Scourian Complex⁵. The Older gneiss samples were supplied by, or collected in collaboration with, Dr F. B. Davies. Almost all analysed samples are one- or two-pyroxene quartzofeldspathic gneisses, some of which exhibit incipient retrogression into amphibolite facies. Several samples contain hornblende, garnet or biotite.

Pb was extracted from whole rock powders by the dissolution-electrodeposition procedure of Arden and Gale⁶. Blanks were between ~0.1 and 0.5% of total Pb extracted, averaging 10 ng per dissolution. Isotopic analyses were made on a 12-inch solid-source mass spectrometer, using silica gel-phosphoric acid activator on single Re filaments. Decay constants for ²³⁸U and ²³⁵U were taken as $1.551 \times 10^{-10} \text{ yr}^{-1}$ and $9.85 \times 10^{-10} \text{ yr}^{-1}$ respectively⁷. (Previous publications

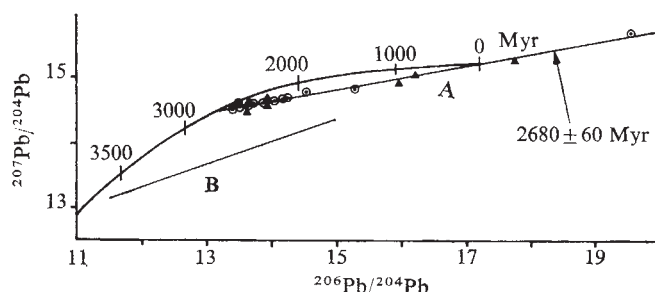


Fig. 1 Pb/Pb plot of analytical data (line A). ○, Older gneiss group of Davies¹; ▲, Scourie gneisses. For comparison, line B is the maximum range of present-day measured values for ~3,700-Myr-old Amitsoq gneisses of West Greenland.

from this laboratory used the older recommended values of $1.537 \times 10^{-10} \text{ yr}^{-1}$ and $9.72 \times 10^{-10} \text{ yr}^{-1}$).

²⁰⁷Pb/²⁰⁴Pb and ²⁰⁶Pb/²⁰⁴Pb ratios are plotted in Fig. 1, line A. The data points define an isochron, but to achieve perfect fit analytical errors must be magnified by three. The isochron yields an age of $2860 \pm 60 \text{ Myr}$ (2σ), which agrees well with other recent age determinations on Scourian rocks⁸⁻¹⁰. The ²³⁸U/²⁰⁴Pb ratio (μ_1) for the source region of the gneisses was 7.7—the value taken for plotting the Pb isotope primary growth curve in Fig. 1. This value is well within the range typical of upper mantle source regions. There is also a good correlation (not plotted) between ²⁰⁸Pb/²⁰⁴Pb and ²⁰⁶Pb/²⁰⁴Pb data, yielding a value of 3.1 for the Th/U ratio of the gneisses (correlation coefficient = 0.97). This is within the characteristic range for many primary igneous rocks.

For comparison, line B in Fig. 1 represents the maximum range of measured present-day Pb isotope compositions for ~3,700-Myr-old Amitsoq gneisses of West Greenland (ref. 11, 12 and unpublished data) which include the most unradiogenic terrestrial Pb isotope ratios yet reported. It is evident that the analysed gneisses (Table 1) contain no detectable Pb derived from a source similar to Amitsoq gneisses. At 2,800 Myr ago, Pb in Amitsoq gneisses was even less radiogenic than today and nowhere near the initial Pb isotope composition of any known Lewisian gneisses (Fig. 1). We believe that this argues strongly against the

Table 1 Analytical data

Sample no.	Locality and grid reference	²⁰⁶ Pb/ ²⁰⁴ Pb*	²⁰⁷ Pb/ ²⁰⁴ Pb*	²⁰⁸ Pb/ ²⁰⁴ Pb*
Older gneiss group ('Uamhaig' gneisses ¹)				
CD1	Badcall Bay, 146418	13.85	14.63	33.86
CD2	Badcall Bay, 146418	19.54	15.73	38.50
CD3	Scourie More, 142442	13.41	14.53	33.15
CD4	Scourie More, 142442	13.48	14.60	33.38
D1	Badcall, 145417	14.82	14.80	35.21
D3	West Scourie More, 140448	14.02	14.65	33.64
D4	Badcall, 145418	15.27	14.85	35.12
D5	West Scourie More, 139448	13.63	14.58	33.24
D7	Upper Badcall, 184443	14.23	14.71	34.33
D12	North Badcall, 152432	14.14	14.70	34.44
CM 1A	Badcall Bay, 146420	13.49	14.58	33.55
CM 1B	Badcall Bay, 146420	13.69	14.63	33.89
Scourie gneisses				
MW 13	Upper Badcall, 161432	15.94	14.96	36.72
MW 14	Upper Badcall, 155417	13.92	14.67	34.09
MW 17	West Scourie More, 145447	16.19	15.05	35.88
MW 20	North-west Scourie More, 142448	13.67	14.60	33.57
MW 22	Scourie Bay, 149449	13.89	14.62	33.92
MW 38	Scourie Bay, 155449	13.89	14.64	33.86
MW 40	Scourie Bay, 152452	13.60	14.50	33.42
MW 41	Scourie Bay, 152452	17.76	15.30	37.05

*Average within-run precision better than 0.1%.

presence of such ancient sialic crust within, or beneath, this part of north-west Scotland. In agreement with earlier work¹³ we postulate that the presumed igneous precursors of Scourian gneisses separated from upper mantle or basic lithosphere not more than ~100–200 Myr before the measured date.

Whilst these findings do not necessarily contradict the field evidence of Davies¹ regarding the relative ages of different gneiss groups, they suggest a much more condensed time sequence possibly similar to that of the Nûk gneisses of West Greenland⁴ and render detailed Archaean correlation between north-west Scotland and West Greenland implausible.

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Giant late Jurassic sabkhas of Arabian Tethys

THE discovery that anhydrite–gypsum evaporites with characteristic textures are forming today in the shallow subsurface of supratidal coastal sabkhas and in continental sabkhas from Arabia^{1,2} has revolutionised the interpretation of ancient evaporite successions. Several ancient evaporite basins, of great economic importance, lately regarded as former hypersaline lagoons or barred basins, have been recently re-interpreted as the deposits of former prograding sabkha plains^{3,4}. In this note we demonstrate that late-Jurassic evaporitic sulphates and minor chlorides of Saudi Arabia, Iran, Iraq, Kuwait, Trucial States, Yemen and Aden were deposited on giant sabkhas (>2 × 10⁶ km²) on the southern margin of the former Tethys Ocean. At their maximum extent in Tithonian times these inferred sabkhas seem to have been among the largest yet discovered. The evaporitic facies throw some light on the evolution of giant sabkha plains. In addition the sabkha plains form the caprocks to some of the world's most prolific oil reservoirs^{5–7}, so that understanding of evaporite genesis is of outstanding economic interest for Middle East stratigraphic studies.

In Saudi Arabia (Figs 1, 2), anhydrite members occur at four main horizons in the Arab and Hith Formations of late Kimmeridgian to Tithonian age^{8,9}. The uppermost Hith anhydrite occurs in Trucial Coast and Iranian sub-crops^{8–10} and finds correlatives in the Gottnia anhydrite of Iraq, Kuwait and north-west Iran^{7,8,11} (Figs 1,2). The Arab–Hith Formations have been regarded as slightly younger than the Amran Group evaporites of Yemen and Aden⁹ but these latter are poorly dated, being overlain conformably by the Upper Tithonian Marine limestones. Faunal

data in ref. 9 cannot rule out the possibility that the Amran evaporites may be late Kimmeridgian to middle Tithonian in age.

Investigations of the Arab–Hith evaporites in the Saudi surface outcrops are hindered by massive Tertiary dissolution, while in the subcrop there are, as yet, only generalised thickness data from oil exploration coreholes¹². At Dahl Hith, however, 32 km south-east of Riyadh, the type section of the Hith anhydrite occurs (Fig. 2) in a deep dissolution cavern where we have recently documented clear evidence that sabkha-type facies are present, with nodular, 'chicken-mesh' aggregates and enterolithic veins in quartz silt-rich, dolomitised grainstone hosts. Crudely layered anhydrite units with disrupted fabrics, resedimented clasts and laterally persistent anhydrite–dolomite varves also occur. The Arab–A carbonate member consists of dolomitised pelletal grainstones showing traces of large scale cross stratification. The Arab–B evaporite member at the base of the section shows particularly fine nodular and layered anhydrite. At outcrop, sections in the basal Arab–D evaporites are rare in the collapse breccia zone, but at Wadi Nisah (Fig. 1) we have found bedded remnants of chicken-mesh anhydrite, now calcitised, in a dedolomitic calcite matrix. Traced eastwards in the subsurface, the Arab

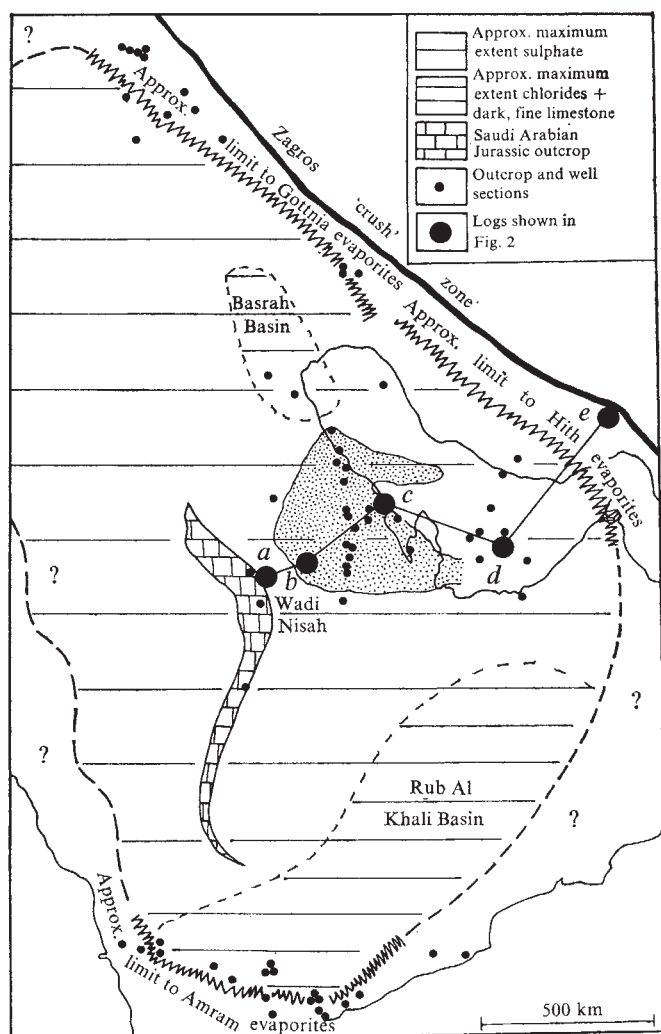


Fig. 1 Map to show principal surface and subsurface sections used to delimit the approximate near-maximum extent of the Arabian late Jurassic sabkhas during Hith Anhydrite times (after data in refs 5–12, 16–19 and unpublished data). Stippled area indicates maximum development of porous lime sands during Arab–D times¹⁶ (see Fig. 2). These now form the major Saudi oil reservoirs.

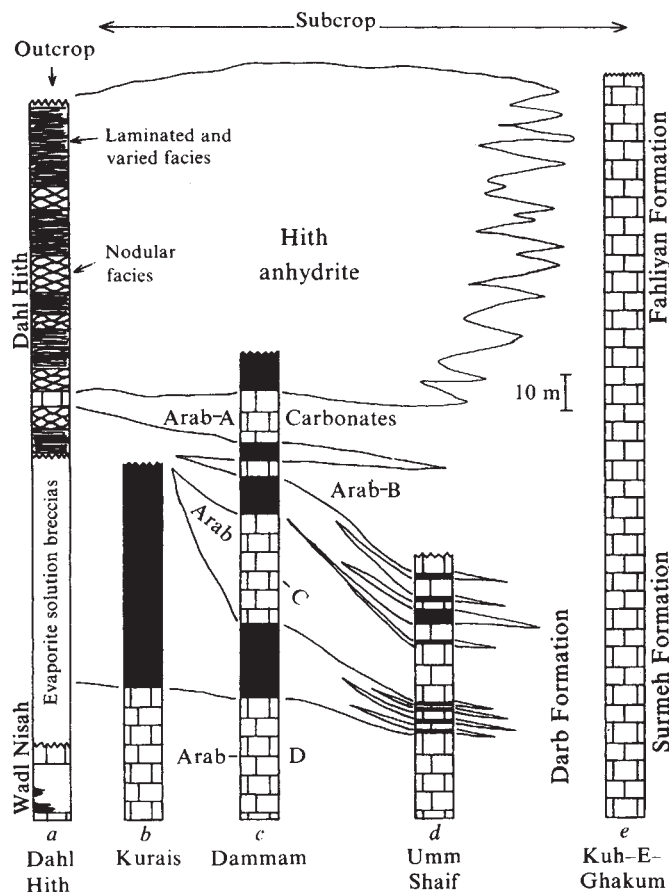


Fig. 2 Major facies changes in the Arab/Hith Formations and their correlatives in the Gulf and Iran to show general eastwards tonguing-out of evaporites away from the landward parts of the supposed sabkhas in central Arabia. Location of sections in Fig. 1. (Data for *a*, authors' results; *b*, *c*, refs 5, 6, 12; *d*, ref. 10; *e*, ref. 8). Note that anhydrite in section *d* is of nodular type only.

Formation anhydrite members thin progressively towards the Persian Gulf while the interbedded grainstones of presumed offshore bar origin tend to thicken^{5,6,12} (Fig. 2) until they thin on to the Surmeh shelf of the south-eastern Gulf and Iran⁶. In the Umm Shaif field of offshore Abu Dhabi the Dharb Formation shows many thin sabkha cycles and is correlated with part of the Arab Formation in the Saudi and Qatar subcrops¹⁰. As the Hith anhydrite is traced eastwards into Iran it is replaced by partly dolomitised offshore marine carbonates of the Surmeh Group⁸ (Fig. 2).

From the above discussion it would seem that sabkha progradation occurred repeatedly from west to east during Upper Kimmeridgian and Tithonian times, that is, from the core of the Arabian shield towards the shelf margin of Arabian Tethys (>1,200 km). Sabkha progradation was followed in each depositional cycle by marine transgression. Sections in the present westernmost outcrops thus contain evaporitic facies precipitated progressively further from the Tethyan coast as time proceeded during each progradational episode. Following Walther's Law, therefore, the vertical facies sequence within each evaporite cycle should demonstrate how evaporitic environments evolved with time. In eastern areas the thin sabkha cycles such as seen in the Umm Shaif field¹⁰ represent more or less precise analogues to the deposits of modern coastal sabkhas which have only been developing since the post-glacial standstill some 4,000–5,000 years BP (ref. 2). In this area repeated transgression followed by carbonate deposition and then sabkha progradation has given rise to many thin sabkha

cycles. To the west, however, in the outcrops of the Arab-Hith Formations at Dahl Hith, there are repeated thicker rhythms of nodular anhydrite, sometimes with signs of a carbonate matrix, followed by laminated and varved anhydrite. It is very noticeable that the carbonate members, dominant in the Arabian-Trucial States coastal areas, thin progressively westwards as well (Fig. 2). Sabkha cycles with hitherto unexplained layered and varved upper members are also recorded from the Permian of Cumbria, England¹³.

We infer that as coastal sabkhas prograde out to some critical width subsurface anhydrite nodule growth ceases and the interior sabkha plains are converted in some way into periodically emergent playas giving rise to laminated and varved anhydrite. Exactly how this process occurs is at present unclear. The evidence of resedimented anhydrite clasts, and buckled and broken anhydrite laminae indicate that the postulated brine pools were frequently dried up. Scattered quartz grains may indicate some aeolian input into the sabkha plains but we stress that the more important features of continental sabkhas as discussed by Kinsman², such as remnant aeolian dune sets, deflation surfaces and adhesion ripples are absent. As Kinsman has noted², a broad prograding sabkha surface will tend to be depressed below the base level of erosion as basin down-warp continues. When, as in the present case, there is an absence of aeolian deposition a broad depression would result which might receive brine input due to (1) seepage from the local brine table, (2) marine recharge, possibly from channel courses linked seawards to marine areas, (3) surface drainage from continental hinterlands, (4) continental brine migration, likely to be most important relatively close to the landward sabkha margin¹⁴. The source of such brines is clearly of the utmost importance and deserves further study. In such interior parts of giant sabkha plains sodium- and potassium-rich bitterns should also occur, their formation being aided by the fractionation process outlined by Smith¹⁵ for the Permian Zechstein evaporites. Potash is not known in the Arabian evaporites, but extensive halite occurs in Kuwait⁷, Aden⁹ and the Saudi Rub al'Khali¹⁶ basin. These areas of chloride precipitation, not usually furthest from the presumed Tethyan shoreline, may represent areas of the Arabian platform undergoing greatest subsidence¹⁶. Associated organic-rich, sometimes foetid micritic limestones lack the well-washed skeletal constituents of the typical Arab Formation carbonates¹⁶ and may be ascribed to rapidly subsiding, possibly partly euxinic, brine-filled basinal areas within the Arabian platform.

As Kinsman² has pointed out, the rate of shoreline regression in the modern Trucial Coast, has been about 1–2 m yr⁻¹ during the past 4,000–5,000 years. Over 10⁶ years a sabkha could develop 1,000–2,000 km wide, as postulated above. A minimum of four periods of major late Jurassic sabkha progradation is recorded in the Arab/Hith Formations in the western Saudi areas. These deposits together probably formed over a total of about 10⁷ yr, so it would be entirely in order for individual sabkha progradation to have occurred over periods of about 2 × 10⁶ yr.

Our palaeogeographical sketch (Fig. 3) shows the maximum likely extent of the giant Arabian sabkhas in relation to a late-Jurassic restoration of the continental plates that formerly bounded the Tethyan ocean. It is clear that the coastal plain was bounded on three sides by areas undergoing normal shallow marine carbonate and clastic sedimentation. Repeated marine transgression over the structurally simple Arabian platform, giving rise to source rocks and potential oil reservoirs, was thus followed at least four times by massive eastward coastal progradation and sabkha evaporite formation during late Jurassic times. Detailed sedimentological analysis of these evaporites will follow in a later contribution.

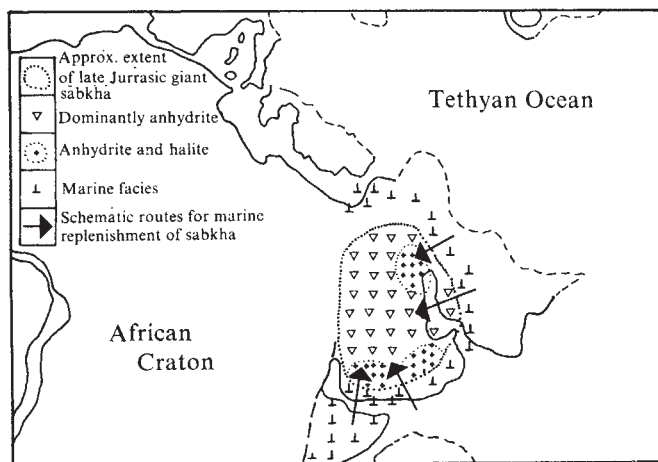


Fig. 3 Maximum likely extent of the Arabian giant sabkha for Hith evaporite times in relation to the distribution of late-Jurassic marine facies on the southern margin of the Tethys ocean. We do not indicate a separate Iranian plate on this sketch. Plate configuration after ref. 20. Additional facies data for East Africa and eastern Mediterranean from refs 21, 22. Position of replenishment arrows entirely schematic.

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Contact angle and film jumps

THE mechanism of capillary rise in a random sphere packing is such that, depending on the absolute value of the wetting- or contact-angle, θ , at equilibrium either a saturation gradient, widest for $\theta = 0$, is observed, or a sharp front between the saturated and the dry part of the porous medium, above some critical value of θ (ref. 1). We present here another partition in the phenomenological description of capillary rise for systems which have $\theta > 0$. There are systems in which θ remains constant and others in which θ decreases during capillary rise. In particular, attention is drawn to an uncommon hypothesis that can explain a decrease in θ . Given the nature of the absorption isotherm in the neighbourhood of saturation, small variations in vapour pressure may induce discontinuous film thickness jumps on a microscopic scale, which may display itself macroscopically as a changing contact angle.

Only capillary rise of organic liquids in random packings of glass beads is considered. For capillary rise of a sharp liquid front in a packing³

$$dh/dt = (k/h)(h_c - h) \quad (1)$$

where h is the height of capillary rise at time t , h_c the (capillarity) driving force, and k the Kozeny–Carman permeability. The value of k depends only on the packing and the viscosity and the density of the liquid. The value of h_c depends on the geometry of the pore space, the liquid surface tension, σ , and the contact angle. Hence, for a combination of a solid packing and a liquid, a straight line should be observed when dh/dt is plotted against $1/h$ —that is, the velocity curve is straight. The value of k can be calculated for random sphere packings. The value of h_c can then be calculated from equation (1). In our experiments⁴ the emphasis was on the variation of many practical relevant parameters, not on the purity or cleanliness of the substances. Capillary rise was observed for n -hexane, n -heptane, paraffin oil (medicinal), toluene, ethanol, n -octanol, glycol, acetone, carbon tetrachloride. α -bromonaphthalene and dichloroacetic acid. Glass spheres of different chemical constitution were used. For some glass surfaces and some liquids θ seemed to be zero. In that case a saturation gradient is formed at equilibrium^{1,5}. Before the saturation gradient is formed, equation (1) is obeyed in that the velocity curve is linear and k agrees with theory, such behaviour is called rapid capillary rise. When $\theta \neq 0$ equation (1) is rarely obeyed. An example is given in Fig. 1 for paraffin oil; when $\theta > 0$ no saturation gradient is formed at equilibrium^{1,5}.

When the velocity curve is concave towards the dh/dt axis, the dh/dt change is slower than equation (1) predicts. This is called slow capillary rise. (Hence 'slow' and 'rapid' do not refer to the absolute value of the capillary rise. A slowly moving viscous liquid such as paraffin oil displays rapid rise. A rapidly moving thin liquid such as hexane may display slow rise, see Fig. 1. Slow capillary rise has been observed for all liquids mentioned above and at some glass surfaces.

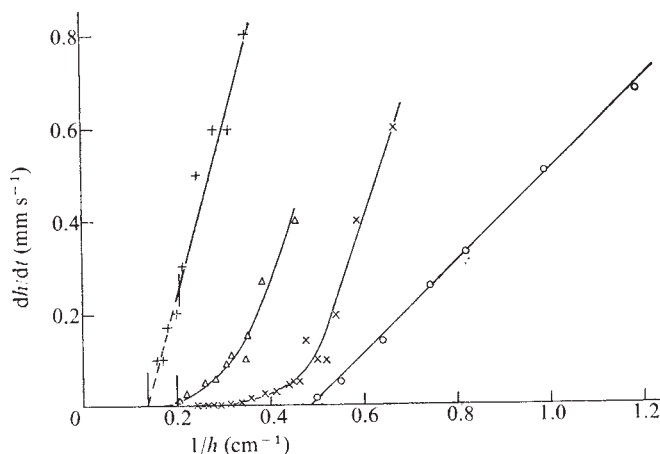


Fig. 1 Capillary rise experiments are carried out by submerging a packing of glass beads 2 mm below a liquid surface, which is kept at a constant level. The packing is contained by a glass tube and supported by a glass filter, or it is slightly sintered, in which case no support is needed. The packing is prepared using a controlled deposition and vibration technique. The homogeneity is checked by means of X-ray absorption. The capillary rise is observed visually or by X-rays. When there is a sharp liquid front both methods give the same result. The packing, X-ray absorption, and sintering techniques have been described in detail elsewhere². The capillary rise of hexane $\circ$, Δ , and paraffin oil $\times$ in a packing of 300–400 μ m sodaglass beads is depicted: $\circ$ and $\times$, rapid rise; Δ and $\times$, slow rise; $+$ and Δ , saturation gradient at equilibrium; $\circ$, sharp front at equilibrium (coordinates on vertical scale have to be multiplied by 100).

Reports elsewhere support the fact that slow capillary rise corresponds to a decreasing contact angle during capillary rise⁴. Although equation (1) applies formally in that the movement of the liquid front is caused by the pressure gradient $(h_c - h)/h$ and constant resistance to flow, k , the observed dh/dt is determined by the change in h_c . We carried out many experiments to check that the slow capillary rise was not due to secondary factors such as a change in packing structure, air entrapment, capillary condensation, or evaporation. Because the geometry is constant, for any liquid k is constant and h_c depends only on the contact angle and the liquid surface tension, σ . Further, it seems impossible that slow rise is due to an increasing σ (θ being constant), because this would imply for the curves of hexane (which has $\sigma = 18 \text{ dyn cm}^{-1}$) that at the beginning of capillary rise σ is about 8 dyn cm^{-1} .

It is not possible to discuss here the numerous hypotheses that might explain a decreasing θ . The following possibilities were considered: unknown compounds on the solid surface, in the liquid, from the air, etc.; heterogeneity of the surface (geometric or energetic); (electro) chemical changes of the surface (penetration, solution); change in film properties (formation, displacement, (selective) evaporation); capillary condensation. It is almost impossible to design experiments that discriminate between these hypotheses. For example, with toluene as a wetting liquid, *inter alia*, the following parameters were varied—glass composition: lead-, soda-, boro-, silicate-, and aluminosilicate-glass; numerous treatments, including vacuum, heat, acids (also hydrogen fluoride), NaOH, detergents; various rinsing and drying methods (including those which yield $\theta = 0$ for water on glass plates); liquid: from different suppliers, degassed, freshly distilled, dried over molecular sieves, dissolved compounds added of higher and lower surface tension and or vapour pressure; vapour: in the absence of air, evaporating from liquid surface, with a gas pressure opposing capillary rise $[(h_c - h) - \Delta P]$ instead of $(h_c - h)$ in equation (1).

Although these variables sometimes had a small effect on the kinetics of rise, in no case was rapid rise observed, in the sense of obeying equation (1). Moreover, an increasing contact angle was never observed (This would be shown by k being higher than the theoretical value). Glass surfaces yielding $\theta = 0$, could be changed (irreversibly) into surfaces yielding $\theta > 0$ and slow rise, by applying surface treatment. Therefore, it is concluded that none of the hypotheses receives significant support. A more interesting but more speculative hypothesis to explain the general phenomena of decreasing contact angles follows.

When $\theta = 0$, at equilibrium, after capillary rise, the adsorbed film, a thin macroscopic wetting film, and the meniscus are in equilibrium. When $\theta > 0$ there is some kind of discontinuity between the bulk liquid and the adsorbed film. Although straightforward and mentioned by Freundlich⁶ in 1922, it is seldom realised that, if $\theta \neq 0$, the adsorption isotherm should cut the $\phi = 1$ axis one or more times (ϕ is the relative vapour pressure, p_v/p_s).

One way to describe the nature of the adsorption film in the region $\phi = 1$ is by using the concept of disjoining pressure⁷, p_d , defined as $l d\mu/dl$, with l the thickness of the film (defined for molecular as well as macroscopic films) and μ the chemical potential per unit volume of adsorbed film. The film is thermodynamically stable if $(\partial\mu/\partial l)_T > 0$. If p_d is positive for all l , $\theta = 0$ for all values of l . If $\theta > 0$, there is a region of unstable film thickness for which p_d is negative. This means that at some vapour pressures, in particular at p_s , different θ , corresponding to different l are possible. Hence, small variations in p_v (or μ for a non-homogeneous multicomponent system) may cause discontinuous changes in l , called film jumps.

Many strange phenomena become more intelligible when it is realised that variations in vapour pressure may cause discontinuous jumps in the film thickness and hence the contact angle. The best examples are found amongst the experiments of Bangham *et al.*⁸—for example θ for a drop of benzene placed on a mica surface decreased when brought under the influence of a jet of supersaturated benzene vapour. Further, it seems

possible that some Marangoni phenomena in which a solid surface is present are in fact caused by film jumps.

During capillary rise small fluctuations in temperature (or concentration of unknown compounds) cause local changes in vapour pressure (or thermodynamic film potential) and therefore possibly film jumps, and hence a change in θ . This causes a minor local increase in capillary rise, which may trigger the nearest cavity (or 'pore', refs 1, 5) to be filled, which gives a minor rise of the whole liquid-vapour interphase. A local increase of θ , will not decrease the macroscopic height of the liquid front, because the curvature at which a cavity drains is much higher than the curvature at which it fills (structural hysteresis). This may explain why until now we have not observed slow rise in cylindrical capillaries. (Not many data are available, however, because nearly always $\theta = 0$.) The fact that paraffin oil does not show the behaviour described may correspond to the observation by Hardy⁹ that there is almost no adsorption film in this case.

Although we realise that we have not given very strong support for the film jump hypothesis, we think it is worth consideration. Wetting phenomena have some role or other in almost any scientific discipline. We would appreciate any information about other phenomena or considerations that have a bearing on this subject.

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Palaeoecological investigations of a site at Hampstead Heath, London

OUR understanding of the vegetational history of Britain is based mainly on studies of peat-bogs, lake and kettlehole infills¹: away from areas where such deposits exist, there is a paucity of information². A multidisciplinary investigation of a deposit associated with a Mesolithic site on Hampstead Heath, North London, is providing rare evidence about man's effect on the vegetational succession of south-east England. The pollen spectrum indicates that *Tilia* rich forest existed until the elm decline, followed by continuing clearance episodes and heath formation. Seeds and beetle remains, which provide more precise information on local conditions, confirm the overall pattern of change from natural forest to cleared areas used for cultivation and grazing. The Mesolithic site of West Heath, Hampstead (TQ2566 8676), is being excavated by the Hendon and District Archaeological Society under the direction of Mr D. Collins³. The site stands on dry, acid Bagshot Sands, but environmental investigations have been carried out at West Heath Spa, a boggy area about 300 m to the south-east, where permanent waterlogging is maintained by a chalybeate spring. At this spot, samples for pollen, seed and insect analyses were collected at 5-cm intervals from below the modern root layer to a depth of 130 cm. Results of these studies are briefly described here.

A pollen diagram (Fig. 1) has been drawn up from

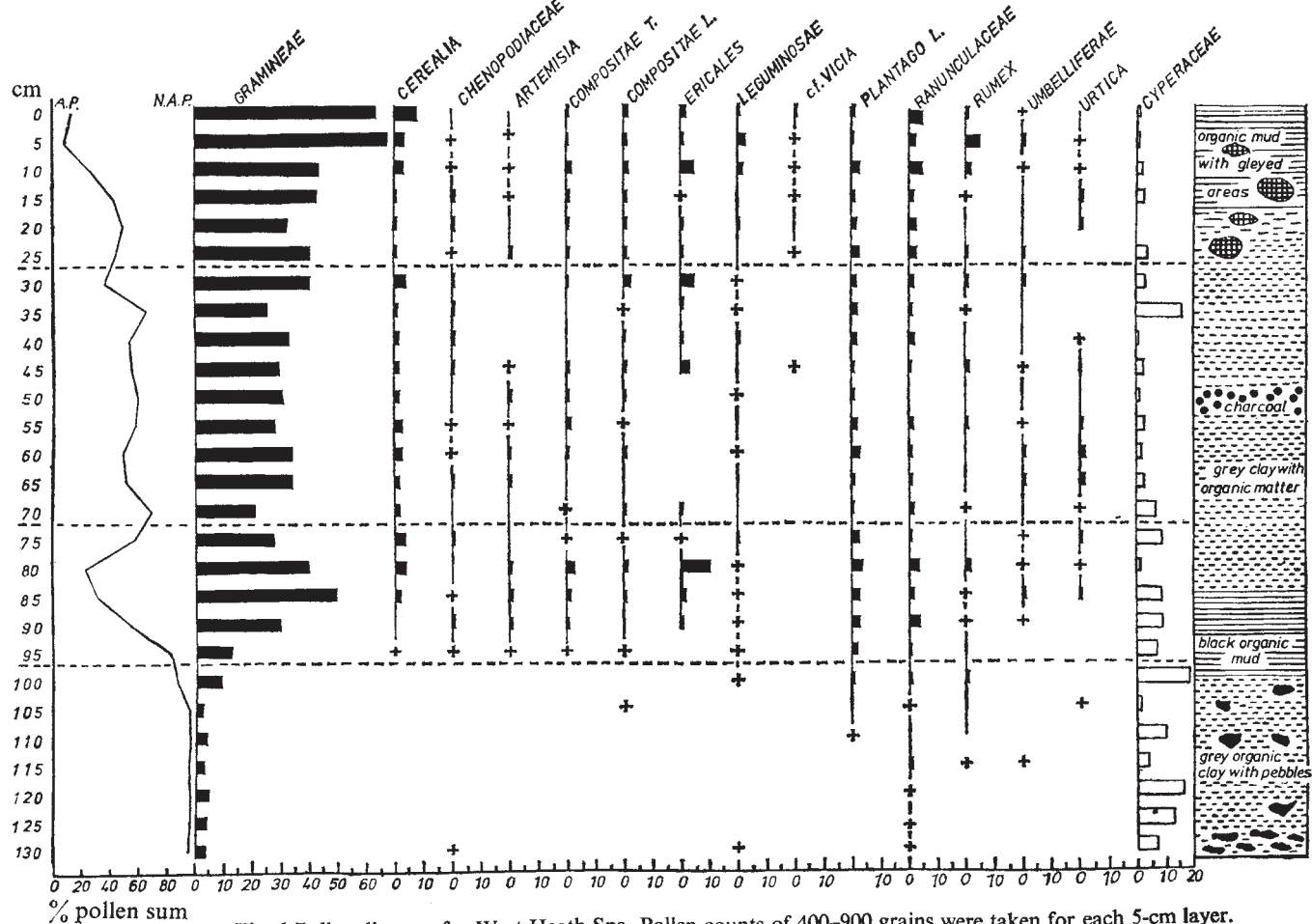
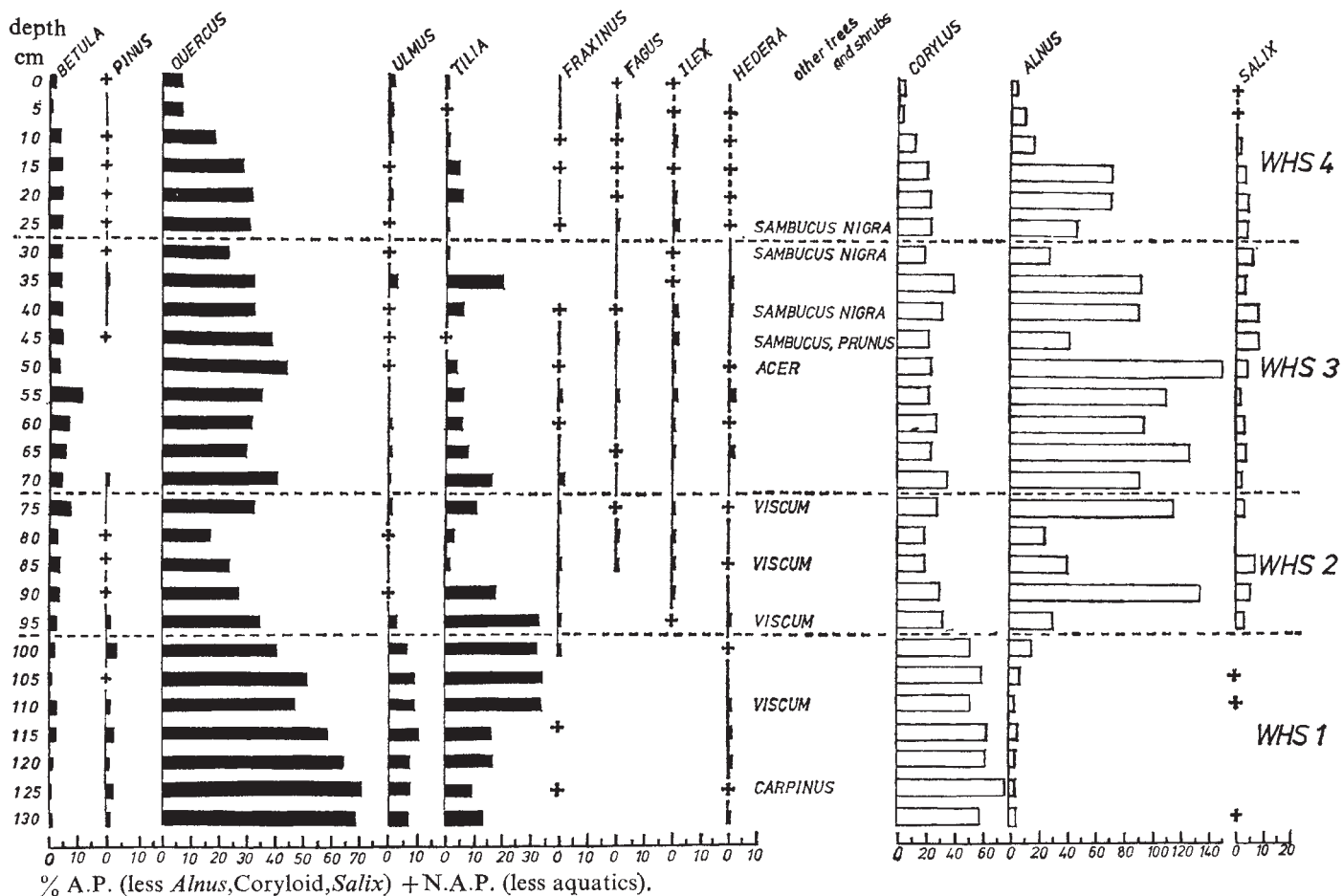


Fig. 1 Pollen diagram for West Heath Spa. Pollen counts of 400-900 grains were taken for each 5-cm layer.

counts of 400–900 pollen grains for each 5-cm layer. It can be divided into four zones of which 130–100 cm (WHS 1) corresponds to Zone VIIa, 100–75 cm (WHS 2) and 75–30 cm (WHS 3) correspond to VIIb and 30–0 cm (WHS 4) corresponds to VIII. These changes are reflected by the seed and beetle assemblages.

Quercus (oak) is the most commonly represented pollen type in WHS 1 but, *Tilia* (lime), an insect-pollinated tree, is believed to be under-represented in the pollen rain⁴, and at West Heath Spa, its substantial pollen record seems to indicate a forest composed predominantly of lime. The abundance of lime pollen, seen also at Shustoke, Warwickshire⁴, Butterbump, Lincoln (J.G., unpublished), and Epping Forest (P. A. Moxey and P. M. Oxford, personal communication) shows that contrary to earlier concepts of mixed oak forest during the Atlantic, lime was probably the dominant forest tree in the Midlands, southern and eastern England. The high values of oak and *Corylus* (hazel) compared with *Alnus* (alder) are probably due to the Spa's situation, an isolated marsh rather than a large wetland with surrounding alder carr. Amongst wood-dependent beetles, the commonest is *Ernoporus caucasicus* Lind., a small lime-feeder which has been found in numbers in deposits of this age⁴, but was first captured in Britain as recently as 1954 (ref. 5). Other scolytids include *Kisso-phagus hederæ* (Schmidt.), an ivy feeder, and the only host specific species in this country⁶, and *Xyleborus xyle-graphus* (Say) which is found on pines as well as several deciduous trees. Also present are the oak leaf-miner *Rhynchaenus quercus* (L.), the holly-feeding cerambycid *Pogonocherus hispidus* (L.) and the small anobiid *Gastrallus immarginatus* (Mull.)—a typical old forest species.

Delineating the boundary between Zone VIIa/VIIb, the pollen values for elm decrease dramatically and appearing for the first time are Cerealia and ruderals such as *Plantago lanceolata* L. (ribwort plantain), which are associated with the activities of man. Accompanying this classic elm decline are falls in the level of oak and lime and *Corylus* pollen. The appearance of *Fagus* (beech) in WHS 2 and maximum frequencies of *Ilex* (holly) in WHS 3 might indicate their expansion into areas of previously closed forest. The appearance of Ericales pollen denotes the start of heath formation. The insect fauna of WHS 2 contains a smaller percentage of tree obligate species than lower samples, although records of decaying wood inhabitants such as *Eremotes ater* (L.) are noteworthy, particularly if, as Rackham suggests, primitive man was unable to clear away large stumps and trunks of felled trees⁷. Both seeds and beetles reflect increased wetness leading to pond formation. Aquatic Ranunculaceae including *Ranunculus flammula* L. and *R. (Batrachium)* spp. are present together with *Isolepis setacea* (L.) R.Br. (bristle scirpus) and *Lycopus europaeus* L. (gypsywort). Seeds of *Pedicularis sylvatica* L. (lousewort) have been recorded for the first time in a Zone VIIb deposit. The beetle fauna is dominated by aquatic species of *Helophorus*, *Hydrochus* and *Hydraena*.

After the elm decline, a semi-stable state is maintained in WHS 3, perhaps by a succession of clearance episodes, the concentration of charcoal suggesting the use of fire. Renewed heath formation is inferred from the reappearance of Ericales. A new faunal element, dung beetles of the *Aphodius*, *Onthophagus* and *Geotrupes* genera, which appears at 80 cm, probably indicates grazing by herbivorous animals in the grasslands.

The final pollen zone, defined by a further steep fall in tree pollen, including *Corylus* and *Alnus*, with a corresponding rise in herbaceous pollen, is characteristic of pollen diagrams from the Iron age onwards. A similar pollen diagram has been drawn up from New Palace Yard, a London site dated to the Iron age (J.G. and S. Limbrey, unpublished). The continuing record of

Gramineae, Cerealia, Leguminosae, *Plantago* and *Rumex* (dock) indicates further cultivation, and Ericales are present throughout. *Sirodendron cylindricum* (L.), is one of the very scarce wood-dependent beetles from these upper samples, most of the species require open land or waterside habitats, such as *Hydrothassa marginella* (L.) and *Plateumaris discolor* (Panz.), a cottongrass-feeder which favours acidic habitats.

Further investigation of all the organic remains at West Heath Spa should provide important evidence on several aspects of the prehistory of the area. It should be possible to determine closely the environment of the Mesolithic habitation site, to investigate man's increasing modification of the landscape after the elm decline and finally, to evaluate the anthropogenic factor in the formation of Hampstead Heath.

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Summer food as the factor limiting roe deer population size

THE sizes of unexploited populations of roe deer (*Capreolus capreolus*) are regulated by spring migration. Individuals which have been able to establish a territory (bucks) or home range (does) force all other animals to move out of the habitat. This phenomenon is specific to age class, and if there is no mortality amongst adult animals, most yearlings emigrate to find new forest area^{1,2}. Thus, in order to develop an intensive harvest policy for roe deer population, knowledge of the number of animals which can be supported by the habitat (that is, social carrying capacity) is essential to avoid undesirable population losses through emigration. During 1970–75, an intensive team research study was carried out in various regions throughout Poland³. Deer food resources were estimated by the harvest plot method³, and winter population densities were precisely determined by drive census techniques¹¹. Based on these data, several study areas were selected in which hunting and natural mortality were very low, and where it was known that most juvenile animals emigrated each spring. We show here that summer food is a major factor limiting roe deer population size.

Census data included the proportion of fawns in the population. Amongst adults, I assumed a constant proportion of 2.01 females per male as determined by Strandgaard¹³. Adding the number of adult males and females killed by hunters, it was possible to determine the density of resident bucks (RB) and resident does (RD) in various forest types. These values were plotted next on log-log scale, against the summer food supply (SF), that is, deciduous browse and dicotyledonous ground flora production, because such components constitute basic summer diet of roe deer¹² (Fig. 1).

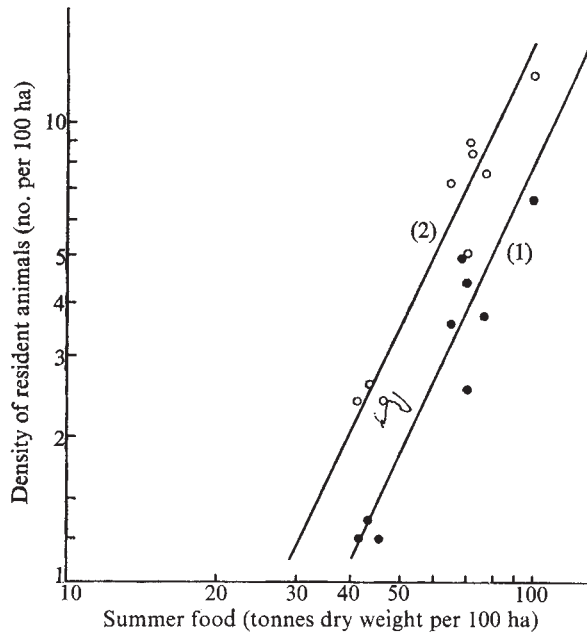


Fig. 1 Influence of summer food supply on the density of resident bucks and does. Based on studies of roe deer in Poland, 1970–75. Curve (1), $Y = 0.00058 X^{2.06}$ (bucks). Curve (2) $Y = 0.00114 X^{2.06}$ (does). $r = +0.93$.

Regression analysis was used to determine the relationship between the $\log_{10}$ of the density of bucks and the $\log_{10}$ of the total biomass of available summer forage (metric tons per 100 ha) for various localities. The correlation was very high ($R = 0.93$, $P < 0.05$) and is described by the formula

$$RB = 0.00058 SF^{2.06} \quad (1)$$

Since the proportion of females is assumed above to be constant in the population, the correlation is also $+0.93$ and the equation determined by least square regression is

$$RD = 0.0014 SF^{2.06} \quad (2)$$

These findings suggest that food resources are an important factor determining the number of resident bucks and does during summer season. This means that animals living in the forest with high availability of summer food, occupy smaller territory size than those inhabiting forest with poor summer food availability. Thus, territory size (TER) or home range (HR) area determines the number of resident animals per unit area; that is, the social carrying capacity of habitat, and this value may be defined by the equation

$$SC = \frac{1}{TER} + \frac{1}{HR} \quad (3)$$

This idea is important because in roe deer the summer availability determines territory or home range size, which then dictates the social carrying capacity of the habitat¹³. This is in contrast to the often held idea that the density of animals influence the territory size.

The territory size is also correlated with the age of roe deer. Using Strandgaard's data on the age of bucks and their territory size, the next relationship was determined¹³

$$Y = 11.02 \text{ age}^{0.616} \quad (4)$$

Using equation (1) and (2), and assuming the same exponent of the age of does in equation (4) to determine their home range, the territory or home range size may be expressed as function of both summer food supply and age of animal

$$TER \text{ (ha)} = 72118.2 \text{ age}^{0.616} SF^{-2.06} \quad (5)$$

$$HR \text{ (ha)} = 36632.6 \text{ age}^{0.616} SF^{-2.06} \quad (6)$$

Table 1 contains comparisons on summer and winter food resources for roe deer in seven common forest types in Europe². Knowing total winter food consumption by a roe deer (56.3 kg dry weight; ref. 14), and using equations (3), (5) and (7), the summer (social) and winter (trophic) carrying capacities of these habitats were calculated.

If winter snow depth did not exceed 15 cm (ref. 2), the determining factor in roe deer population density is the summer carrying capacity, expressed by variation in territory size.

Table 2 Roe deer population density and average number of corpora lutea per adult female in different parts of Europe

Population density (Animals per 100 hectare) (X)	Corpora lutea per adult female (Y)	Country
13.0	2.00	Denmark (data from ref. 8)
13.3	1.85	Poland (ref. 4)
13.4	1.89	England (ref. 10)
14.9	2.18	England (ref. 10)
28.6	1.90	Poland*
56.0	2.40	Denmark (ref. 13)
63.0	1.90	Denmark (ref. 13)
63.0	2.00	Denmark (ref. 1)

$Y = 1.95 + 0.0022X$; $R = +0.27$, $P > 0.05$; corpora lutea maximum : minimum = 1.3.

* B.B., unpublished.

Except the alder forest and possibly, the fresh deciduous forest type (fresh referring to mesic soil conditions), the winter carrying capacity has no effect on the density of roe deer populations. If one season reproduction results in a doubling of the population size, the winter forage can potentially limit the density in alder and fresh deciduous forest (see Table 1).

As a consequence of territoriality, roe deer possess a density-independent reproductive rate. There was a positive correlation between population density and the average number of corpora lutea per female (Table 2), but this correlation is not statistically

Table 1 Natural food resources of roe deer, their average territory and home range size, as well as summer and winter carrying capacity of some European forests

Forest ecosystems	Food-tons dry weight per 100 hectare		Territory size of bucks (hectare)	Home range of does (hectare)	Carrying capacity of forest (no. per 100 hectare)	
	Summer	Winter			Summer	Winter
Fresh coniferous	40.4	13.9	69.6	35.4	4.3	246.9
Fresh mixed coniferous	83.3	8.6	15.7	8.0	18.9	152.7
Moist mixed coniferous	64.3	6.6	26.7	13.6	11.1	117.2
Mixed deciduous	70.4	3.4	22.2	11.3	13.3	60.4
Mountain deciduous	30.0	6.1	128.5	65.3	2.3	108.3
Fresh deciduous	72.9	1.5	20.6	10.5	14.4	26.6
Alder	112.4	2.0	8.5	4.3	32.5	35.5

The data are based on studies in Poland in 1970–75.

significant ($R = +0.27$, $P > 0.05$). It is well known that non-territorial populations of white-tailed deer (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*) show a significant negative correlation between population density and reproductive rate. The average number of corpora lutea per female decreases as mule deer population increases ($R = -0.98$ (ref. 7)). Also, the same strong negative correlation was shown for white-tailed deer, regarding ova ($R = 0.92$) and embryos ($R = 0.90$) produced per female⁷. The ratio of maximum/minimum value of corpora lutea per female (a measure of variability) was recorded as 1.9 for mule deer⁹ and 3.1 for white-tailed deer⁵, but was only 1.3 in roe deer populations (Table 2).

Such small variability of number of corpora lutea per roe deer female, and the lack of correlation between density and reproductive rate probably result from spring social regulation of the population. As the territory is established to provide an individual with an adequate food resource, each individual of the same age possesses a territory large enough to provide equal amounts of summer food. Evidence which supports this thesis is the fact that the number of corpora lutea in a fenced roe deer population dropped drastically to 1.6, while free living roe deer in the same vicinity showed 2.1 per female⁸.

Although this study was initiated to facilitate the intensive management of European roe deer populations, the results presented here have clear implications for an understanding of the natural regulation of populations of large herbivores.

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Injury requirement for initiation of regeneration of newt limbs which have whole skin grafts

THERE are three known requirements for salamander limb regeneration—*injury*, *nerves*, and *wound epidermis*^{1,2}. During the past few years we have examined cellular events in amputated, regenerating limbs and compared these with non-regenerating limbs after either denervation^{3,4} or after whole skin grafts over the amputation surface⁵. Results of these experiments led to the formulation of an hypothesis⁶ which suggests unique roles for injury, nerves, and the wound epidermis during the initiation of regeneration: injury is important to cause dedifferentiation of limb stump tissue cells, entry of these cells into G₁ of the cell cycle and DNA replication; nerves are important for one or more G₂ events and thus indirectly for mitosis; and the wound epidermis maintains these cells in the cell cycle so that significant outgrowth (blastema formation) can occur. In the present study we have examined the extent and kinds of injury necessary to initiate regeneration in newt limbs

which have had skin grafts for 5 weeks and externally show no regeneration. The results suggest that non-cycling dedifferentiated cells are present in 5-week skin graft limbs which immediately begin cycling when a wound epidermis forms after re-injury.

Whole skin was grafted over the amputation surfaces of 57 adult newt (*Notophthalmus viridescens*) limb stumps immediately after amputation of the limbs through the proximal portion of the radius and ulna as previously described⁵. Newts remained anaesthetised on moist gauze for 2 h and were positioned so that pressure from the gauze enhanced healing of the graft. Five weeks after amputation and skin graft, 44 of these limbs showed no external signs of regeneration. The other limbs either regenerated normally (six cases) or regenerated at a right angle from the area of skin flap overlap (seven cases) as seen in previous experiments.

These 44 non-regenerating limbs with skin flaps were either maintained as controls without re-injury or injured by: (1) a fresh amputation 1 mm proximal to the original amputation, (2) careful removal of the entire skin flap with watchmakers' forceps in order to expose the original amputation area in its entirety, and to allow new wound epidermis to form, (3) careful removal of a third of the original amputation surface to expose an area equal to only a third of the original amputation surface, (4) 15 penetrations through the skin flap with a sharp, sterile needle to a depth of 1 mm into the stump tissue, or (5) cutting a single transverse incision across the limb stump with a sterile razor blade to a depth of 1 mm into the stump tissue. We attempted to make these incisions parallel with the plane of the skin flap but they occasionally varied by as much as 20°.

The results of these various methods of injury can be seen in Table 1.

Table 1 Regeneration responses after re-injury of non-regenerating newt limbs containing skin grafts*

Type of re-injury	Number of skin graft limbs	Regeneration	
		Yes	No
Control: no re-injury, skin graft remains intact	6	0	6
Re-amputate 1 mm into stump	8	8	0
Remove entire graft to expose entire amputation surface	8	7	1
Remove 1/3 of graft to expose 1/3 of amputation surface	5	4	1
15 needle penetrations 1 mm into stump	8	0	8
Transverse razor incision to a depth of 1 mm	9	8	1

*Limbs with skin grafts were examined 5 week after grafting. Only those which showed no regeneration were re-injured. Limbs were observed externally for regeneration at weekly intervals for 4 week.

Of the various methods used to re-injure skin graft limbs, all elicited regeneration responses with the exception of the 15 needle penetrations (Table 1). It is important to note that control amputations, removal of the entire skin graft, and removal of $\frac{1}{3}$ of the graft, all resulted in wound epidermis formation over the amputation surface, albeit only $\frac{1}{3}$ of the area in the latter case. When $\frac{1}{3}$ of the skin graft was removed, the wound area did not increase in size. Instead, regeneration occurred from the small wound surface and a small regenerate with apparently normal morphogenesis was the result.

It is surprising that 15 needle penetrations through the skin flap did not result in regeneration. Since the needle penetrations were to a depth of 1 mm into the stump, clearly some injury of stump mesodermal tissues occurred. The 15 individual wounds either did not retain free wound epidermis or the small wound epidermis areas were

Table 2 Enhanced regeneration of skin graft limbs re-injured by transverse incision compared with control amputated limbs*

Type of re-injury	Week of observation	Pre-mound	Stages of regeneration reached			
			Mound	Cone	Paddle	Digit
Transverse incision	1	3	6	0	0	0
Amputation	1	9	0	0	0	0
Transverse incision	2	2	3	2	2	0
Amputation	2	9	0	0	0	0
Transverse incision	3	1	1	3	3	1
Amputation	3	4	5	0	0	0
Transverse incision	4	1	0	3	4	1
Amputation	4	0	6	3	0	0

*Only 5-week skin graft limbs which showed no external signs of regeneration were used for these re-injury experiments. The transverse incision was made to a depth of 1 mm into the stump. Skin grafts were made only on left limbs at which time right limbs were not amputated. Thus at 5 weeks, right limb controls were amputated for the first time. All control limbs and eight out of nine re-injured skin graft limbs eventually completed regeneration. Newts were maintained at 21 ± 1 °C throughout the experiment.

insufficient for regeneration to occur. A complete histological study of these re-injured limbs now in progress in our laboratory should clarify this problem.

Regeneration after re-injury of skin flap limbs could conceivably require dedifferentiation of stump tissues as well as wound epidermis formation⁶. According to the cell cycle hypothesis of Tassava and Mescher⁶, the wound epidermis prevents re-differentiation of dedifferentiated stump tissue cells. In skin flap limbs, Mescher⁵ found dedifferentiation, DNA synthesis, and mitosis 1 week after amputation to be equal to that of control amputated limbs with wound epidermis. However, by 2 weeks these parameters decreased significantly in skin flap limbs but increased in control limbs. Tassava and Mescher⁶ and Mescher⁵ suggested that in the absence of the wound epidermis, cells left the cell cycle and re-differentiated. The present results indicate that complete re-differentiation of limb stump cells does not occur in skin graft limbs, at least not during the 5 weeks following grafting. Particularly significant in this regard are the results of the transverse incision re-injury (Tables 1 and 2). In these limbs, a ridge formed along the incision within 4 d of re-injury in eight out of nine cases and mound blastemas were present by 1 week. From Table 2 it can be seen that some of these regenerates reached cone and even paddle stages of regeneration by 2 weeks after re-injury and digit stages by 3 weeks. Regenerates of control amputated limbs lagged far behind (Table 2). It is important to note that even at 26 °C, control newt limbs do not reach paddle or even cone stages by 2 weeks post-amputation⁷. In the present study, regeneration stages were also reached very early after entire or $\frac{1}{3}$ skin graft removals, but accurate staging data was not obtained.

What is likely, therefore, is that a significant number of dedifferentiated cells are present in skin flap limbs which are not traversing the cell cycle and thus are not seen as mitotic figures and do not label with ³H-thymidine⁵. Thus when a wound epidermis forms after re-injury, these cells immediately begin cycling and dividing. Regeneration of control limbs is therefore slower because of the time required for dedifferentiation. Our preliminary histological observations are consistent with this view. It should now be possible to determine which stage of the cell cycle these dedifferentiated cells are in and whether, after even longer post-graft times, that is 8–10 weeks, similar re-injuries will elicit regeneration. These latter experiments are particularly important since Polezhaev⁸ observed no regeneration when whole skin grafts were removed from axolotl limb stumps after an 8–9 week graft period. Re-injury of newt limbs with 10-week-old skin grafts will test whether dedifferentiated cells are still present and capable of blastema formation.

The regenerates which developed after a transverse incision is made through the skin flap always formed in the plane of the incision. Since some of these incisions varied by as much as 20° from the normal dorsal ventral

axis of the limb, the wound epidermis seems to have a strong directional influence on regeneration, consistent with Thornton's⁷ early findings. Additional experiments further varying the axis of the incision, and removing varying areas (and shapes) of skin grafts, will provide additional insights into the role of the wound epidermis in regeneration.

The results of these experiments confirm Mescher's⁵ results that cell dedifferentiation initially occurs in skin graft limbs in the complete absence of a wound epidermis but DNA synthesis and mitosis then subside without outgrowth. These cells apparently remain dedifferentiated for 5 weeks since rapid blastema development is seen after re-injury and wound epidermis formation. These findings are consistent with the views presented by Tassava and Mescher⁶ that the wound epidermis is not required for dedifferentiation but that it is important to keep dedifferentiated cells moving through the cell cycle.

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Bilateral interaction in olfaction

THERE has been much interest in perceptual interactions between the ears and between the eyes, but much less in those between the nostrils, or more precisely, the two sides of the olfactory system^{1–4}. This report reveals that, for a wide range of supraliminal stimulation, there is considerable interaction between the two sides. The facets considered here are bilateral additivity of odour intensity, and bilateral influence of an adapting stimulus presented unilaterally.

In the experiment on bilateral additivity, 20 subjects estimated the intensity of various concentrations of *n*-butyl alcohol presented to either or both nostrils. There were six stimuli—five concentrations of odorant prepared in aqueous solution plus plain water. Samples (1 ml) were placed in small vessels designed for olfactory testing and for ready assessment of vapour phase concentration⁵. Concentrations of odorant, assessed chromatographically, varied from 0.10 to 7.60 mg l⁻¹ of air in a geometrical progression. On each trial, the subject sniffed the contents of two vessels simultaneously, one placed at the right and

the other at the left nostril. On the first trial, both vessels contained 0.86 mg l^{-1} . The subject was instructed to assign the number 10 to the odour magnitude produced on this trial. On subsequent trials, the subject judged various left-right and right-left combinations formed from the six concentrations, including water. Of the 36 possible combinations only one, water-water, was excluded. The subject assigned numbers proportional to odour magnitude. Thus if a particular combination smelled three times as strong as the first, the subject called it 30, and so on (method of magnitude estimation)⁸. Order of presentation was irregular. Each subject served in two sessions, identical except for order of presentation.

The results, averaged geometrically across subjects, yielded a family of psychophysical functions relating odour intensity to concentration. Isointensity contours constructed from this family offer a way to decide on the degree of bilateral interaction and to compare the results reported here with analogous results in other modalities (Fig. 1)^{2,7}. The contours show the combinations of concentrations to the right and left nostrils that yielded each of several odour intensities, $\psi = 5$ to $\psi = 35$.

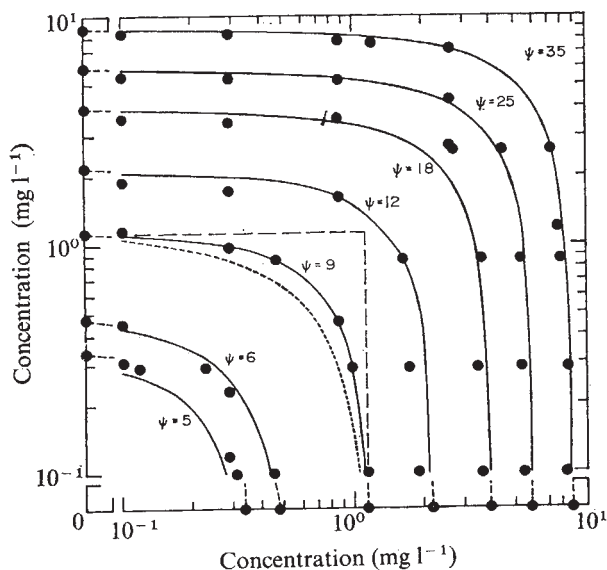


Fig. 1 Data points show the various combinations of concentrations in the right and left nostrils that led to equal perceived intensity (ψ) at various levels ranging from 5 to 35. The dashed rectilinear contour exhibits the shape that would have resulted if the perceived magnitude of a bilateral complex had equalled the magnitude of the higher concentration of the complex smelled alone. The dotted contour exhibits the shape that would have resulted if the perceived magnitude of the complex were governed by the total amount of stimulus, irrespective of how it was distributed between the nostrils. The solid contours depict the vector sum of the mass of odorant in the right and left nostrils (see equation (1)). The angle between the vectors was 60° .

The shapes of the contours showed that olfaction behaves neither like audition, where the loudness of a binaural complex falls close to the sum of the loudnesses of the monoaural components, nor like vision, where the brightness of a binocular complex characteristically falls below the brightness of the stronger monocular component^{8,9}. Other straightforward possibilities merited consideration. If the perceived magnitude of the odour complex had equalled the magnitude of the higher concentration of the complex smelled alone, the contours would have a rectilinear shape (see dashed contour in Fig. 1). Alternatively, if the perceived magnitude of the complex had been governed by the total amount of stimulus, irrespective of how it was distributed between the nostrils, the contours would have the shape shown by the dotted contour. The outcome fell between these two possibilities, but resembled

more closely the dotted contour, which portrays stimulus summation.

Perfect stimulus summation can be described by the equation

$$\varphi_{ab} = (\varphi_a^2 + \varphi_b^2 + 2K\varphi_a\varphi_b)^{1/2} \quad (1)$$

where φ_{ab} is the mass of odorant in the complex, φ_a and φ_b are the masses of the components, and where K equals 1.0. Values of K less than 1.0 reflect degrees of partial summation. To decide whether partial summation could account for the degree of bilateral additivity, the data in Fig. 1 were used to solve for K . The value obtained, 0.5, gave rise to the solid contours. These offer a good account of the results. Accordingly, the olfactory system behaved as if the effective magnitude of the stimulus in a bilateral complex were the resultant of the vector sum of the magnitudes in the components. A K of 0.5 implies a 60° angle between the vectors.

The rule of partial summation exhibited here suggests that when there is an imbalance of stimulation to the nostrils, the side receiving the stronger stimulus makes a disproportionate contribution to total intensity. This is seen in the shapes of the isointensity contours. When the magnitude of stimulation is strong on one side and weak on the other, a gradual increase in the weaker stimulus requires at first only a small compensatory decrease in the stronger in order to maintain perceived intensity constant. As the weaker stimulus approaches the magnitude of the stronger, however, the compensatory decrease becomes considerably larger, and indeed very large when the balance of stimulation shifts from one side to the other. Such behaviour could reflect inhibitory interaction between the olfactory bulbs, a process already seen electrophysiologically and one invoked to account for why the course of adaptation in rats is slowed when the anterior commissure between the bulbs is transected¹⁰⁻¹².

Evidence for bilateral interaction in olfactory adaptation has come also from studies of thresholds in human subjects¹³⁻¹⁵. Specifically, an adapting stimulus presented unilaterally serves to raise the absolute threshold for both ipsilateral and contralateral nostrils. But, the ipsilateral side shows greater and longer lasting loss of sensitivity than the contralateral side. These results implicate bulbar, or other central neural structures, in the process of adaptation¹⁶. It has yet to be shown, however, that the ability of an adapting stimulus to transfer its influence contralaterally occurs on the supraliminal level and with a brief monorhinal adapting stimulus that eliminates the possibility of retronasal leakage of odorant to the contralateral side. To check these possibilities, 30 subjects judged the intensity (magnitude estimation) of various concentrations of linalyl acetate presented after an adapting stimulus to the nostril ipsilateral or contralateral to the test nostril. On each trial, the subject sampled the contents of two vessels successively, but during the same continuous inhalation (4 s duration). Both adapting and test stimuli were presented on the same inhalation in order to eliminate any backflush of odorant to the contralateral posterior naris. Both nostrils were always open, thereby insuring a continuous flow of air from the anterior to the posterior nares throughout inhalation. The first vessel presented on a trial could contain just the diluent (diethyl phthalate) or linalyl acetate (0.53 mg l^{-1} , the adapting stimulus). The second could contain any of five test concentrations (0.02 – 1.60 mg l^{-1}). The first vessel could be presented to either the right or the left nostril, and the second could be presented ipsilaterally or contralaterally with respect to the first. The various combinations of right-left, ipsilateral-contralateral, diluent-test stimulus, adapting stimulus-test stimulus were intermingled randomly within a session. The subjects waited approximately 1 min between trials.

Figure 2 shows the psychophysical functions obtained (1)

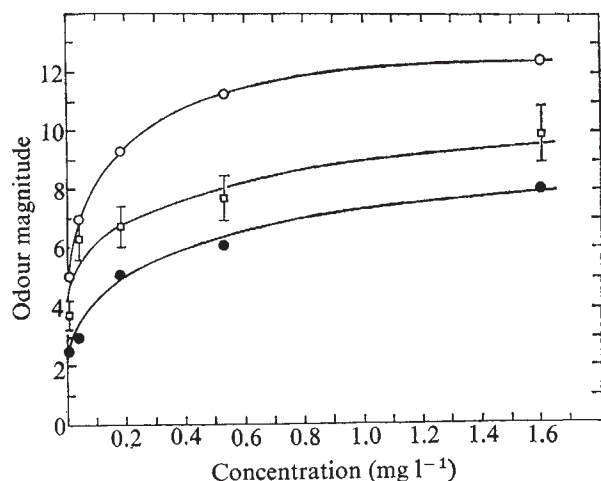


Fig. 2 Psychophysical functions for linalyl acetate obtained when test concentrations succeeded the diluent (no adaptation, ○) and when they succeeded an adapting stimulus (0.53 mg l^{-1}) on the same side (ipsilateral adaptation, ●) or on the opposite side (contralateral adaptation, □). The vertical bars represent the standard error of the difference between the results for no adaptation and contralateral adaptation (upper bars) and between the results for contralateral adaptation and ipsilateral adaptation (lower bars).

when the test stimuli succeeded the diluent, (2) when they succeeded linalyl acetate (0.53 mg l^{-1}) contralaterally and (3) when they succeeded the same concentration of linalyl acetate ipsilaterally. The functions line up in a manner consistent with bilateral interaction. Ipsilateral adaptation was characterised by a marked decrease in perceived magnitude throughout the entire range of concentrations; contralateral adaptation was characterised by a somewhat less marked, but still substantial, decrease. The difference between the two adaptation curves reflects in part the degree of peripheral neural involvement in the adaptation process. But the difference presumably reflects also any attenuation in the effectiveness of the adapting stimulus as its influence transfers contralaterally at some point in the central nervous system.

In summary, there is considerable bilateral interaction in the olfactory system. The interaction is revealed in bilateral additivity of intensity and in adaptation. The interaction seems to be independent of level of stimulation, for the same equation, based on vector addition, can describe the degree of bilateral additivity at both high and low levels of perceived intensity. The equation implies partial summation of stimulus magnitude and suggests that the nature and degree of bilateral interaction in olfaction is unique among the senses.

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Bicyclic phosphorus esters increase the cyclic GMP level in rat cerebellum

4-ALKYLDERIVATIVES of 1-phospha-2,6,7-trioxabicyclo[2.2.2]octane-1-oxide (PTBO derivatives) are highly toxic organophosphates. On intraperitoneal injection to mammals they produce tonic-clonic convulsions and death within a few minutes^{1,2}. Their mechanism of action is unknown, but it is different from that of other toxic phosphorus esters which act as inhibitors of cholinesterase³. The symptoms of poisoning by PTBO derivatives indicate an activation of the central nervous system². There is evidence suggesting that the cyclic nucleotides AMP and GMP act as second messengers in central and peripheral synapses and so we have investigated the effects of PTBO compounds on the level of these nucleotides in the brain. The ethyl (EPTBO) and isopropyl (IPTBO) compounds, two of the most toxic PTBO derivatives, were used. The results show that following intraperitoneal injection of these compounds into rats, the cyclic GMP concentration is increased in cerebellum by both convulsive and subconvulsive doses, whereas the levels of the nucleotide are unchanged in cerebral cortex and subcortical tissues. The increase in cyclic GMP level in the cerebellum is thus not due to the convulsive state.

The PTBO derivatives were synthesised at FOA 4 according to the method of Wadsworth and Emmons⁴. The LD₅₀ value (intraperitoneal, rats) of EPTBO was 1.1 mg kg^{-1} and that of IPTBO 0.1 mg kg^{-1} which is in agreement with values reported by others². Atropine sulphate 20 mg kg^{-1} injected intraperitoneally 20 min before EPTBO did not change the toxicity of the convulsant. Diazepam 5 mg kg^{-1} administered similarly 10 min before EPTBO increased the LD₅₀ value to 2.5 mg kg^{-1} .

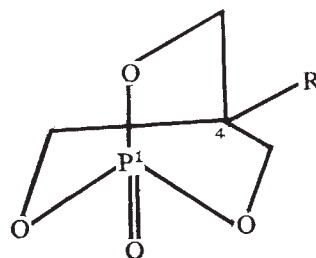


Fig. 1 4-Alkyl-1-phospha-2,6,7-trioxabicyclo[2.2.2]octane-1-oxide. EPTBO: R = ethyl; IPTBO: R = isopropyl.

Cyclic nucleotides were assayed in the brains of Wistar male rats (160–300 g) injected intraperitoneally with PTBO compounds and of controls injected with 0.9% NaCl. The rats were killed by microwave irradiation (2,000 W) for 10 or 40 s for cyclic AMP and cyclic GMP respectively. Animals injected with convulsive doses (EPTBO, 1.2 mg kg^{-1}) were killed during the third tonic convulsion (about 15 min after injection). Control animals and rats given subconvulsive doses (IPTBO, 0.05 mg kg^{-1}) were killed after the same time. In some experiments diazepam (5 mg kg^{-1}) or atropine sulphate (20 or 50 mg kg^{-1}) were injected intraperitoneally before PTBO. Tissue pieces from cerebral cortex, subcortical tissues and cerebellum were homogenised at 4°C and extracted according to Folbergrova⁵. Cyclic AMP and cyclic GMP were assayed using test kits (Radiochemical Centre, Amersham, England).

Table 1 Effects of EPTBO on the levels of cyclic AMP and cyclic GMP in different brain areas

	Cyclic AMP pmol per mg wet weight			Cyclic GMP pmol per mg wet weight		
	Control	During convulsions	P	Control	During convulsions	P
Cerebral cortex	0.32±0.05 (17)	0.34±0.11 (13)	n.s.	0.089±0.013 (6)	0.105±0.014 (6)	n.s.
Subcortical tissues	0.40±0.08 (15)	0.45±0.15 (11)	n.s.	0.137±0.013 (5)	0.187±0.032 (6)	n.s.
Cerebellum	1.29±0.21 (21)	1.74±0.27 (15)	n.s.	0.593±0.054 (8)	2.708±0.338 (8)	<0.001

Values represent the mean±s.e.m. for the number of animals given in parentheses.

P values were obtained by *t*-test; n.s. not significant.

Convulsive doses of EPTBO caused no significant differences in the levels of cyclic AMP in any of the brain areas studied compared with control animals (Table 1). The level of cyclic GMP was, however, increased almost fivefold (470% of the control values) in the cerebellum of rats with PTBO-induced convulsions whereas it was unaffected in the other brain areas studied (Table 1). A subconvulsive dose of IPTBO increased the level of cyclic GMP in cerebellum almost twofold (167% of the control values) (Table 2). Pretreatment with atropine sulphate did not significantly alter the effect of the PTBO derivatives on the cyclic GMP level. Diazepam given 10 min before the administration of the PTBO derivatives, however, blocked the increase of cyclic GMP induced by the subconvulsive dose of IPTBO and reduced the increase by the convulsive dose of EPTBO from fivefold to less than twofold (Table 2).

The elevation of cyclic GMP in the cerebellum seems not to be due to the convulsive state of the animals since a subconvulsive PTBO dose also caused an increase. The cyclic nucleotide content of postsynaptic cells in neural tissues is regulated by transmitters released from nerve terminals impinging on the cells^{5,6}. Increase of cyclic GMP in the brain might be correlated to stimulation of the muscarinic cholinergic receptor (mAChR), but atropine, a well known mAChR blocker did not reduce the IPTBO-induced increase of cyclic GMP level in the cerebellum. In addition, atropine neither affected the convulsions nor decreased the toxicity of the PTBO compounds and preliminary results showed no effect of PTBO derivatives on acetylcholine release from cerebral cortex. This seems to rule out interference with muscarinic cholinergic neurotransmission.

The symptoms of PTBO poisoning are similar to those produced by bicuculline and picrotoxin, known blockers of γ -aminobutyric acid (GABA) receptors. Bowery *et al.*⁷ have reported that PTBO compounds act as specific antagonists to the action of GABA. In addition we have found a slightly reduced K⁺-induced release of ³H-GABA from synaptosomes in the presence of PTBO derivatives⁸. GABA is the inhibitory transmitter released at many synapses in

the cerebellum⁹. The PTBO-induced increase of cyclic GMP in the cerebellum might be caused by primary action of these agents on the inhibitory GABA mechanism. In support of this is the fact that the effects of the PTBO derivatives on the cyclic nucleotides—an increased cyclic GMP level in cerebellum and unaffected cyclic AMP—are in accordance with those described for some agents interfering with the GABA-ergic mechanism^{10,11} and also that diazepam, suggested to exert a facilitatory effect on GABA transmission¹², decreased both the PTBO-induced elevation of cyclic GMP and the toxicity of these compounds.

The Purkinje cell axons of cerebellum participate in the regulation of motor activity initiated in cerebral cortex. Cyclic GMP is involved in the regulation of Purkinje cell activity and elevation of cyclic GMP in the cerebellum has been suggested to occur in those cells⁹. The cyclic GMP concentrations are suggested to be regulated by the interaction of excitatory and inhibitory transmitters⁹. Blocking of GABA action would increase the release of excitatory transmitters. The putative cerebellar transmitters glutamate and noradrenaline have both been reported to stimulate cyclic GMP synthesis in cerebellum^{9,13}. The increase of cyclic GMP induced by the PTBO derivatives may be an expression of an increased release of excitatory transmitters from neurones synapsing on the Purkinje cells.

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Table 2 Effects of atropine and diazepam on the PTBO-induced increase of cyclic GMP in cerebellum

Treatment	Cyclic GMP (pmol per mg wet weight)
Saline	0.593±0.054 (8)
IPTBO 0.05 mg kg ⁻¹	0.989±0.048 (6)
Atropine 50 mg kg ⁻¹ + IPTBO	0.963 (2)
Atropine 20 mg kg ⁻¹ + IPTBO	0.958 (2)
Diazepam 5 mg kg ⁻¹ + IPTBO	0.576±0.084 (4)
EPTBO 1.2 mg kg ⁻¹	2.708±0.338 (8)
Diazepam 5 mg kg ⁻¹ + EPTBO	1.048±0.082 (4)

Atropine 20 mg kg⁻¹ and 50 mg kg⁻¹ given intraperitoneally 15 and 30 min respectively before injection of IPTBO (0.05 mg kg⁻¹). Diazepam (5 mg kg⁻¹) was similarly injected 10 min before IPTBO (0.05 mg kg⁻¹) or EPTBO (1.2 mg kg⁻¹). Controls received 0.9% NaCl. Values are means ± s.e.m. for the number of animals given in parentheses.

A new class of GABA agonist

COMPOUNDS which mimic the activity of the inhibitory neurotransmitter γ -aminobutyric acid (GABA) on post-synaptic receptors are of particular pharmacological interest as possible therapeutic agents in human neurological disorders and as molecular probes with which to study different types of GABA receptors. The GABA molecule has considerable flexibility with free rotation around all three carbon-carbon bonds as indicated in Fig. 1. This conformational mobility is reduced in GABA analogues such as trans-4-aminocrotonic acid¹ and muscimol², which are potent GABA agonists at bicuculline-sensitive receptors

on spinal neurones of the cat. The conformational mobility of such compounds can be reduced still further by incorporating the amino function in a ring structure. This has led to a new class of GABA agonist described here, based on 1,2,3,6-tetrahydropyridine-4-carboxylic acid (isoguvacine) and the related bicyclic isoxazole 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP) (Fig. 1). Isoguvacine represents a semi-rigid analogue of trans-4-aminocrotonic acid in a folded conformation. THIP, a folded analogue of muscimol, is even more rigid as the 'masked' carboxyl group (the 3-isoxazolo moiety) is fixed in the general plane of the molecule. The action of these compounds as GABA agonists provides indirect evidence that GABA interacts with bicuculline-sensitive postsynaptic receptors in the cat spinal cord in a partially extended and almost planar conformation.

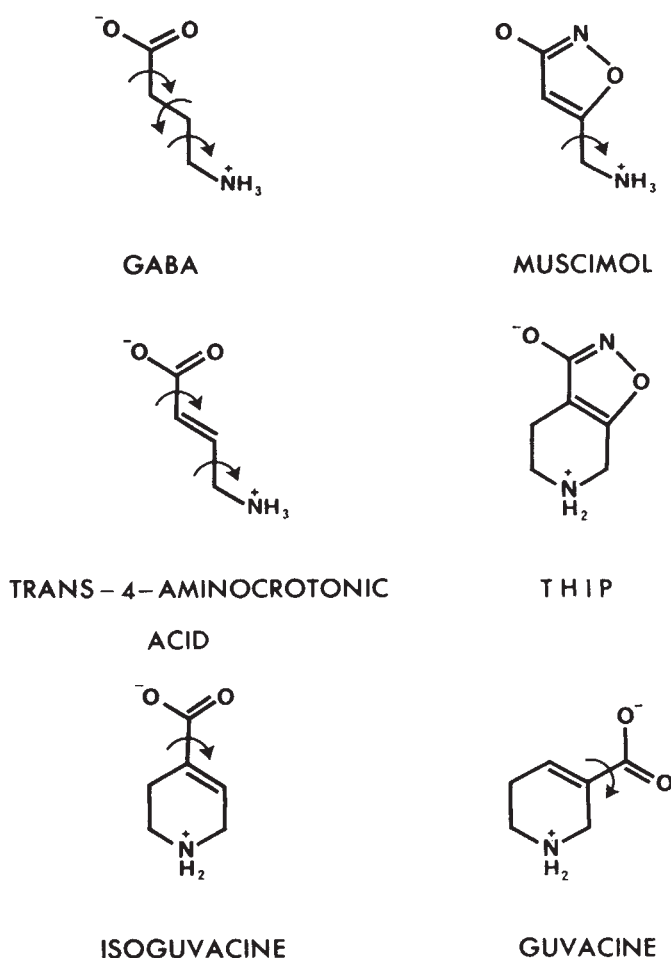


Fig 1 Conformational mobility of GABA and related compounds as illustrated by the rotational freedom around carbon-carbon single bonds marked with arrows.

Experiments were performed on lumbar dorsal horn interneurons and Renshaw cells of three cats anaesthetised with pentobarbitone sodium (35 mg per kg body weight intraperitoneally, supplemented when required). Extracellular action potentials were recorded by means of the centre barrel of seven barrel micropipettes and the compounds tested were administered electrophoretically from the outer barrels of the micropipettes², which contained 100 or 200 mM aqueous solutions adjusted to pH 3 except for bicuculline methochloride (BMC, 10 mM in 165 mM sodium chloride) and DL-homocysteate (DLH, 200 mM, pH 7.5). The approximate potency of any

depressant action was assessed relative to that of GABA on the basis of electrophoretic currents required to produce equal and submaximal inhibitions of firing induced by DLH. *In vitro* experiments were carried out to determine the uptake of GABA (10 nM) by small slices (about $0.1 \times 0.1 \times 2$ mm) of rat cerebral cortex³, and the transamination of GABA catalysed by extracts of rat brain mitochondria⁴. The syntheses of the new compounds isoguvacine and THIP will be described elsewhere. Muscimol and BMC were prepared by published procedures^{5,6}. All other compounds were purchased from commercial suppliers.

The depressant action of isoguvacine (tested on 10 neurones) was comparable with that previously reported for muscimol², and was two to four times more potent than that of THIP (eight neurones) and GABA which were approximately equipotent. The depressant action of all of these compounds was reversibly antagonised by bicuculline methochloride in conditions where the inhibitory action of glycine was unaffected (Fig. 2). The saturated analogue of isoguvacine, piperidine-4-carboxylic acid, also depressed the firing of spinal interneurons (3) with a potency similar to that of GABA, and this action was also reversibly antagonised by BMC.

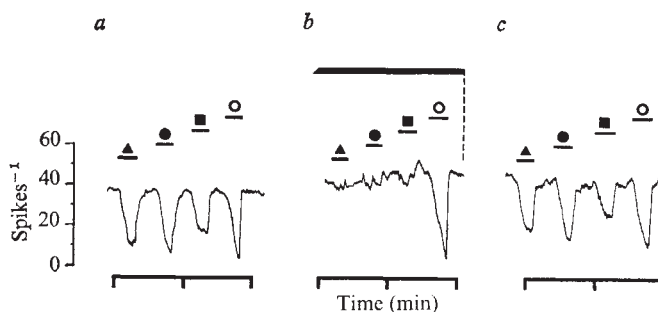


Fig 2 The effect of bicuculline methochloride (BMC) on the inhibition of the firing of a spinal interneurone by isoguvacine (35 nA; ●), THIP (60 nA; ▲), GABA (60 nA; ■), and glycine (15 nA; ○) ejected for the times indicated by the symbols and horizontal bars. The firing of the neurones was maintained with continuously ejected DL-homocysteate (DLH, 10 nA). The relative effectiveness of the inhibitory amino acids is similar to that observed in all experiments. *a*, Before; *b*, during BMC (15 nA) which was terminated at the vertical broken line; *c*, 3 min after (*b*).

As with the development of inhibitors of the neuronal uptake of GABA such as nipecotic acid⁷ and guvacine⁸, the present investigation arose from studies of the structure-activity relationships of isoxazoles related to muscimol. Isoguvacine and THIP were inactive at 0.5 mM as inhibitors of the neuronal uptake of GABA by slices of rat cerebral cortex, and also at 1 mM as inhibitors of the transamination of GABA catalysed by extracts of rat brain mitochondria. It is of interest to contrast isoguvacine and guvacine (Fig. 1) with respect to bicuculline-sensitive GABA receptors on spinal neurones and to transport carriers mediating the neuronal uptake of GABA in brain slices: isoguvacine is apparently a potent agonist with respect to these receptors and inactive against the carriers, whereas guvacine seems to be inactive as a GABA agonist⁹ and a potent substrate-competitive inhibitor of the carriers⁸. These results are in agreement with the proposal that these particular postsynaptic receptors and transport carriers for GABA exhibit different structural specificities¹⁰: isoguvacine and guvacine may represent two different 'active conformations' of GABA selectively interacting with the bicuculline-sensitive postsynaptic receptors and neuronal uptake carriers respectively.

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Abnormal adenine metabolism of erythrocytes in Duchenne and myotonic muscular dystrophy

DUCHENNE muscular dystrophy (DUD) and myotonic muscular dystrophy (MYD) are inherited as sex-linked recessive and autosomal dominant traits, respectively. Although the primary manifestation is progressive muscle weakness, multiple organ systems seem to be affected. Cardiac abnormalities and mental retardation are described in both conditions, and defects in immunoglobulins and endocrine functions have been reported in MYD¹. The primary inherited metabolic defect is unknown, but there is evidence to suggest an abnormal sarcolemmal membrane¹. The possibility that muscle disease, especially involving membrane dysfunction, is reflected in the erythrocytes is supported by the demonstration of defective phosphorylation in the erythrocytes of patients with DUD² and MYD³. In DUD the erythrocytes appear abnormal in the scanning electron microscope⁴ and in MYD electron spin resonance reveals abnormal membrane fluidity. There are other reasons to study erythrocytes, for the direct study of muscle is hampered by the need for repeated biopsies and by atrophy and fibrous tissue, which can cause secondary biochemical changes. Furthermore, the yield of isolated sarcolemmal membranes is often small and impure. In DUD, there is a 30% reduction in muscle ATP⁶ and an abnormal ATP:ADP ratio of 1.57. Whether this is a significant defect or is a result of secondary changes in the muscle has yet to be clarified. We report here that the intracellular phosphorylation of adenine nucleotides

derived from adenine incorporated by the erythrocytes is abnormal in both DUD and MYD.

Ten DUD patients from 10 families, five DUD carriers (two obligate, two probable, one possible) from five families, and seven MYD patients from five families were used and compared with 16 normal subjects, eight patients with other neuromuscular diseases, and four patients with a variety of disorders. 2.5 ml of heparinised venous blood was obtained from each patient in double blind conditions and spun at slow speed (800 r.p.m. 12-inch diameter rotor) for 17 min at 20 °C. The platelet-rich-supernatant plasma (PRP) was removed and separately centrifuged at 3,000 r.p.m. for 5 min to obtain platelet-poor-plasma (PPP). The red cell fraction after removal of PRP was centrifuged at 3,000 for 5 min to pack down the cells. 0.2 ml of packed red cells drawn from the centre of the sample was mixed with 0.2 ml of their own PPP in a polyethylene tube at 37 °C in a water bath. 10 µl of U-¹⁴C-adenine (1 µCi) was added and incubation continued with gentle shaking for 30 min. The tubes were cooled briefly in ice and 0.5 ml of cold 13% perchloric acid (PCA) was added. After mixing and centrifuging at 3,000 r.p.m. the clear PCA supernatant (15 µl) was chromatographed on thin-layer cellulose sheets using a solvent of formic acid-teramyl alcohol-water (10:15:5). After drying each run was cut into 1-cm strips and the radioactivity determined on a liquid scintillation spectrometer⁷. Each peak was calculated as a percentage of the total ¹⁴C-nucleotide pool formed during the incubation period and the profile of peaks run was summarised as a ratio $R = (ATP + ADP)/AMP$ which describes the relative proportions of high-and-low energy compounds. All assays were performed within 2 h on fresh erythrocytes without freezing or storage.

The results on red cells suspended in their own plasma showed significant difference in the ratio of ATP+ADP to AMP of the patients, when compared with normals and disease control (Table 1). The mean ratio (R) for the normal subjects and disease controls were 9.6 ± 4.1 and 8.7 ± 4.6 . MYD patients showed the greatest reduction in their ratio, with a mean of 2.9 ± 1.8 but there was also a significant reduction in DUD (4.9 ± 2.9). Thin-layer chromatography usually demonstrated a reduction in the proportions of both ATP ADP and an increase in AMP production. ¹⁴C-adenine was present in all cases in excess so that it was not rate limiting, but the data suggested that adenine uptake was reduced in the DUD and MYD patients.

ATP exerts a protective effect on cell membrane integrity⁸. Thus any condition which leads to a decreased proportion of intracellular ATP is likely to result in leakage of enzymes and other proteins. As well as acting as a reserve of energy, ATP may have a direct effect in maintaining the integrity of the cell membrane, since it is concerned in the biosynthesis of phosphatidic acids, intermediates in the synthesis of phospholipids in membranes⁹.

Our findings are indicative of appreciable metabolic disturbances in red blood cells of MyD and DuD, which are apparently insufficient to produce gross physiological

Table 1 Mean per cent of ¹⁴C-adenine nucleotide pool in RBCs

	No. of patients	ATP	ADP	AMP	$R = \frac{(ATP + ADP)}{AMP}$	P*
Normal controls (NC)	17	66 ± 13	14 ± 6.1	10 ± 4.0	9.4 ± 4.1	—
Disease controls (DC)	12	66 ± 8.2	16 ± 8.1	14 ± 10	8.7 ± 4.6	NS
Duchenne (DUD)	10	46 ± 19	22 ± 5.6	19 ± 12	4.9 ± 2.9	0.005
Myotonic (MYD)	7	46 ± 14	21 ± 5.4	29 ± 11	2.9 ± 1.8	0.005
Duchenne carrier (DUD C)	5	57 ± 22	8 ± 1.6	22 ± 23	5.1 ± 2.6	NS

NS, not significant. Data are given $\pm$ s.d.

*P determined by Student's *t* test.

dysfunction and therefore may be a useful partial model for the further study of these genetic disorders.

The concept of failure to maintain a normal profile of adenine nucleotides may be an important factor in the membrane defect in Duchenne and myotonic dystrophy and needs further investigation.

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Amino acid sequence similarities between amyloid P component C1t and CRP

THE P component is a normal 9.5S serum α 1 glycoprotein composed of subunits that is found as a minor constituent of all types of amyloid deposits^{1,2}. Painter *et al.*³ have described a 9.5S α 1 glycoprotein, C1t, composed of ten 23,000 molecular weight (MW) subunits, which may have an important role in the regulation of complement C1 function. Ultrastructural and immunological studies showed a striking resemblance between P and C1t (refs 3, 4). CRP is an acute phase reactant, present in many species which can interact with pneumococcal C-polysaccharide, choline phosphatides and various polycations to activate the complement cascade^{5,6}. It is composed of a 23,000 MW subunit non-covalently linked as a pentamer⁷. A clear-cut structural relationship between C1t and CRP has been demonstrated by comparing amino terminal sequences⁷. Since all three molecules—P, C1t and CRP—have a similar ultrastructure, namely, a cyclic pentamer or a decameric double, the

term 'pentraxins' has been proposed by Osmand *et al.* for this group of molecules⁷. We report here the characterisation and the amino terminal sequence of human P component. The P component is identical to C1t except for the absence of the first residue (His), thus providing additional evidence for a close evolutionary relationship for P, C1t and CRP.

P component was isolated by saline extraction, Sephadex, diethylaminoethyl and cellulose chromatography, according to the method of Skinner *et al.*², from the liver of a patient (WIL) with secondary amyloidosis. By sodium dodecylsulphate-polyacrylamide gel electrophoresis, the material consisted of a single 23,000 MW component which had a tendency to aggregate in reducing conditions. Figure 1 compares the amino acid sequence of the first 27 residues with those of C1t (ref. 7), three partially sequenced P components^{2,4}, and CRP (ref. 7). When compared to C1t, our preparation is one residue shorter at the amino terminal. The amino terminal residue could not be identified but the remaining 25 residues were identical. Position 19 is tentative since tyrosine was identified by thin layer chromatography and histidine by amino acid analysis after back hydrolysis. Differences between our P component and those reported previously^{2,4} may be due to individual differences between different preparations or perhaps, in several instances, due to carry-over or other technical problems in residues that are difficult to identify. A closer similarity between C1t and P than between these molecules and CRP is suggested by the finding that both anti-P and anti-C1t antibodies gave a reaction of identity with P, as shown previously⁴, while there was no antigenic relationship between P and CRP. Our anti-P antibody did not react with various preparations of CRP and four antibodies to native and denatured CRP did not react with P (WIL).

These results raise the possibility that there is a series of structurally closely related proteins which may have an important role in regulating and modulating certain immunological and inflammatory reactions⁷. Interestingly, our tissue-derived P component seems to lack the first residue, perhaps as a result of proteolytic cleavage. The structural and antigenic identity observed between C1t and P suggests a closer relationship between them than with CRP. But we do not yet have sufficient data to conclude that they are identical. Study of the interaction of P with antigen antibody complexes, complement components

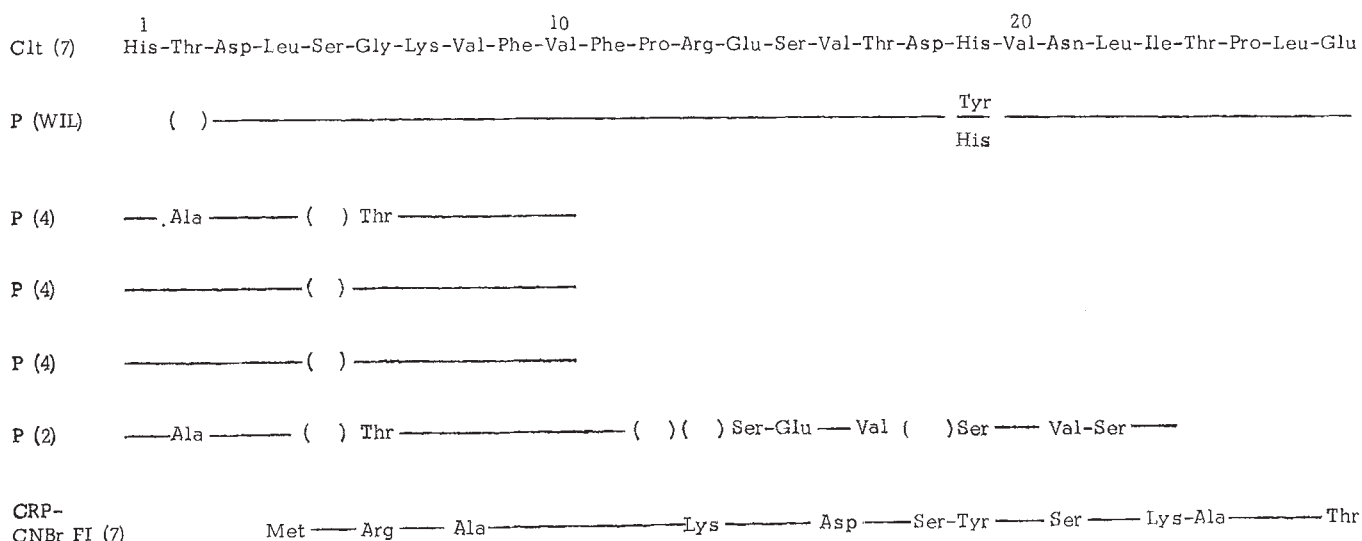


Fig. 1 The amino terminal sequence of human P compared with previously published sequences of P (refs 2, 4), C1t (ref 7) and a cyanogen bromide fragment of CRP (ref. 7). Numbering and homology are based on the sequence of C1t. Amino acid sequence analysis was performed by using a Beckman 890C sequencer and the Beckman DMAA peptide program. Identification of amino acids was done by three methods: (1) gas-liquid chromatography on a 762 0A Hewlett-Packard gas chromatograph⁸; (2) thin-layer chromatography (TLC) of PTH-amino acids on 5 × 5-cm polyacrylamide plates in two dimensions⁹; (3) amino acid analysis using HI for hydrolysis¹⁰. Sequence identities are indicated by —. Unidentified residues by (). Residue 19 yielded tyrosine on TLC and histidine on back hydrolysis. In our conditions of amino acid analysis, PTH tyrosine is often lost and may elute with histidine.

and certain other substances (Y.L., I. Gigli, B.F. and E.C.F., in preparation), as well as further amino acid sequence studies will allow a more precise structural and functional comparison with C1t.

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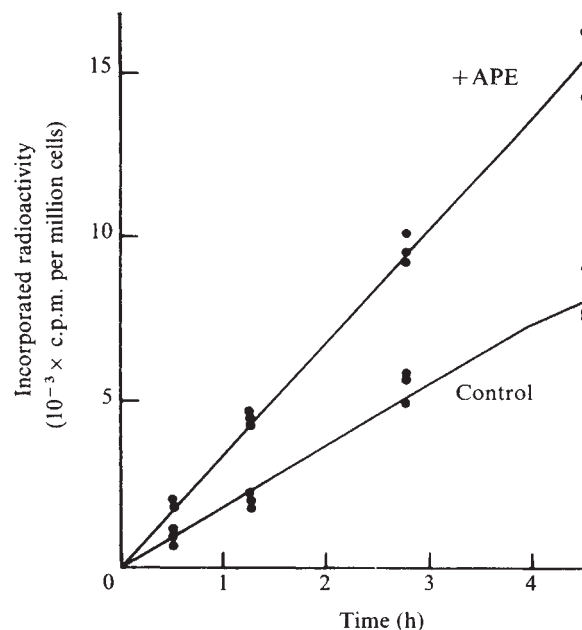


Fig. 1 Effect of anterior pituitary extract (APE) on ^3H -thymidine incorporation in isolated thymocytes. APE was the 2000g supernatant of rat anterior pituitary homogenates in GS medium (glucose salt medium, containing 120 mM NaCl, 5 mM KCl, 5 mM Na_2HPO_4 , 1 mM MgSO_4 , 0.8 mM CaCl_2 , 5.5 mM glucose and 5 mM Tris-HCl buffer, pH 7.2). Rat thymocytes ($15 \times 10^6 \text{ ml}^{-1}$), were incubated in GS medium at 37°C with ^3H -thymidine ($0.5 \mu\text{Ci ml}^{-1}$, 2 μM) $\pm$ APE (added to a final concentration of 0.25 anterior pituitary equivalents per ml of final incubation medium). At different time intervals, the incorporation reaction was stopped by chilling and by adding excess (0.5 mM) of non-radioactive thymidine to the incubation tubes. Cells were centrifuged (600g for 10 min), washed with fresh GS medium, followed by two washings with cold 10% trichloroacetic acid and two with methanol. The pellet was dried and dissolved in 0.1 ml Soluene, transferred to the scintillation vials with a toluene-based scintillation mixture and the incorporated radioactivity measured in a Packard liquid scintillation spectrometer.

An anterior pituitary factor stimulates thymidine incorporation in isolated thymocytes

A POSSIBLE tropic role of pituitary on thymus has been suggested by several studies. Hypophysectomy causes an atrophy of the gland^{1–4} and thymus is poorly developed in Snellbagg genetically dwarf mice with hypopituitary function⁵. A diminution of thymus size and a reduction in immunological responses to T cell-dependent antigens is also seen after administration of anti-hypophyseal serum⁶. The pituitary may contain several active principles affecting directly or indirectly the thymus functions. Of these, growth hormone has been shown to have a promoting effect on metabolic activities of the thymus and on immune responses involving the participation of thymus-derived T cells^{4,7,8}. Thyrotropin, via the thyroid hormones, also exercises a thymotropic action⁹. ACTH, via corticosteroids, causes the transient diminution of the gland¹⁰. We report here the presence in pituitaries of a previously unknown small molecular weight peptide(s) which stimulates markedly the incorporation of tritiated thymidine into DNA in isolated thymocytes.

Figure 1 shows that the addition of the anterior pituitary extract (APE) to the isolated thymocytes resulted in a notable stimulation of the incorporation of ^3H -thymidine into cold trichloroacetic acid (TCA)-precipitable pellet. A stimulation with APE was consistently obtained but the extent varied from preparation to preparation of APE and thymocytes. Thymocytes from young rodents gave a better response than those from aged animals. Table 1 shows the order of variation in stimulation by APE of ^3H -thymidine incorporation in thymocytes obtained from six different animals. Variance analysis of the data indicates that between-animal variations are significantly more marked than between tube variations (variance ratio $F = 29.9$, 8.5 times more than required for significance at 1% level). These variations probably arise due to different internal environments to which the thymocytes are exposed before the animals were killed. The effect of APE is highly

significant ($F = 1163.5$) and is much more than the animal-to-animal variations (variance ratio 159.2 times higher than that required for 1% significance level).

The incorporated radioactivity was sensitive to DNase but not to RNase treatment. Viability of cells was measured at each time point by Trypan blue exclusion and no significant difference was noted in control and APE added tubes.

To identify the pituitary hormones that may be responsible for this action of APE, rat growth hormones (NIAMD-rat GHB1), luteinising hormone (NIH-LH-S 17), follicle-stimulating hormone (NIH-FSH-S 9), thyrotropin (NIH-TSH-B 4), prolactin (NIH-P-B 3) and ACTH (pork ACTH, Chemical Lab. Frideriksberg, Denmark) were tested singly and in combination. Two hormone combinations were prepared: in mixture A the hormones were present at their approximate physiological level in pituitary and in mixture B, at five times higher concentration. These hormones caused a marginal increase in thymidine incorporation, but the effect was not comparable with that obtained with APE (Table 2). The active principle in the extract thus seems to be different from these hormones.

The factor was dialysable and heat stable and was distinct from LH and TSH releasing hormones. The activity was not present in hypothalamic extracts or in posterior pituitary and cerebral cortex extracts. Cyclic nucleotides and inorganic ions did not give parallel stimulation of this metabolic activity in isolated thymocytes. The stimulatory effect was not due to the possible supply of essential

Table 1 Effect of anterior pituitary extract (APE) on ^3H -thymidine incorporation in thymocytes from six young rats

Rat	Body weight (g) and sex	Cell density in assay medium (million per ml)	Incorporated radioactivity c.p.m. per million cells $\pm$ s.e.m.		% Of control
			Control	+ APE	
1	94.5 (F)	4.40	2652 $\pm$ 133 (3)	7086 $\pm$ 174 (4)	266.5
2	77.6 (F)	4.52	1832 $\pm$ 55 (4)	4483 $\pm$ 210 (4)	244.7
3	74.3 (M)	5.12	2189 $\pm$ 28 (4)	5451 $\pm$ 169 (4)	249.0
4	88.7 (F)	3.88	2964 $\pm$ 80 (4)	6553 $\pm$ 84 (4)	221.1
5	69.1 (F)	4.80	2824 $\pm$ 64 (4)	6041 $\pm$ 295 (4)	213.9
6	44.1 (M)	1.76	2184 $\pm$ 42 (4)	6696 $\pm$ 195 (4)	306.6

Thymocytes prepared separately from six young rats were incubated at 37 °C for 2 h $\pm$ APE (0.8 anterior pituitary equivalent per ml) in GS medium containing 1 $\mu\text{Ci ml}^{-1}$ ^3H -thymidine (1 μM). Values in parenthesis are the number of replicates for each preparation of cells.

nutrients by the APE, and was also obtained when experiments were performed in tissue culture media RPMI and Medium 199.

The stimulatory activity of the APE on thymocytes was sensitive to hog intestinal peptidase (from Sigma, containing general proteolytic and aminopeptidase activity) but not to DNase, RNase and trypsin. The factor may therefore be a peptide. Its molecular weight (MW) is 500, and the isoelectric pH 7.9. Purified preparations of the factor do not show significant ultraviolet absorption above 230 nm. These properties indicate that this factor is distinct from other factors of pituitary origin described in recent years by other investigators. Fibroblast growth factor from bovine pituitaries¹² has a MW of 13,300. Chondrocyte mitogenic factor¹³ is a heat-labile entity and was detected as a contaminant in anterior pituitary hormone preparations. The factor influencing the ovarian cells in culture¹⁴ is a non-dialysable protein. Lipotropins¹⁵ are big polypeptides (6,000 and 11,400 molecular weight). A hypophysial factor influencing the steroid metabolism in rat hepatoma cell lines¹⁶ is a high molecular weight entity and in contrast to present case, is present only in female pituitaries. Pituitary endorphins¹⁷ are polypeptides of 3,000–3,500 MW.

The action of APE on uptake and incorporation of thymidine seems to be tissue specific. Thymocytes show maximal stimulation but lymphoid cells derived from spleen and lymph nodes are also stimulated, though to a lesser extent. APE did not have stimulatory effect on thymidine incorporation in tissue slices from liver, kidney, heart and diaphragm.

The biological role of this factor(s) is not fully known. It may influence the proliferation of cells within thymus. Mouse embryonic thymus cultures in media containing this factor show higher cellularity than parallel control cultures.

Thymus is an organ characterised by a high rate of cellular proliferation, which is independent of the antigenic stimulus¹¹, and not much is known on the factors regulating

the metabolic activities and the rate of cell proliferation in this tissue. The size of this gland shrinks with hypophysectomy^{1–4}. The gland is also atrophied in states of hypopituitary function⁵. It is thus conceivable that pituitary exercises an effect on lymphoid cell proliferation in thymus. The presence of a factor exerting a marked influence on DNA synthesis in thymocytes is thus of interest.

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Table 2 Effect of anterior pituitary hormones and anterior pituitary extract on the incorporation of thymidine into isolated thymocytes

Addition	Incorporated radioactivity c.p.m. per million cells Mean $\pm$ s.e.m.	% Of control
None	429 $\pm$ 31 (4)	100.0 $\pm$ 7.2
Hormone mixture (A)	536 $\pm$ 42 (6)	124.9 $\pm$ 9.8
Hormone mixture (B)	584 $\pm$ 57 (5)	136.1 $\pm$ 13.3
Anterior pituitary extract (APE)	1567 $\pm$ 95 (5)	365.3 $\pm$ 22.1

Five million rat thymocytes in 1 ml GS medium, were incubated with tritiated thymidine (0.5 $\mu\text{Ci ml}^{-1}$, 2 μM) with or without hormones/APE at 37 °C for 2 h in a Dubnoff metabolic shaker. Hormone mixture (A) contained at final concentration, 25 μg of GH, 20 μg each of TSH, FSH, LH and prolactins and 10 μg of ACTH per ml of incubation medium. Mixture (B) had five times the concentration of these hormones. APE was added at one rat anterior pituitary equivalent per ml of final incubation medium. Figures in parenthesis give the number of replicates in each case.

Propranolol increases binding of thyrotropin to thyroid membranes

β -ADRENERGIC antagonists such as propranolol possess two pharmacologically relevant properties— β -adrenergic blockade is observed at relatively low concentrations, whilst at higher concentrations they are effective membrane stabilising agents^{1,2}. The latter property which, unlike β -adrenergic antagonism is non-stereospecific, is also described as local anaesthetic or quinidine like activity and it possibly accounts at least in part, for the anti-arrhythmic properties of propranolol—local anaesthetics and quinidine also showing anti-arrhythmic activity^{3–5}. A wide variety of effects have been associated with local anaesthetic activity¹, but as yet there is no unifying explanation of the mechanism of action. We have investigated the effect of propranolol, acting in its capacity as a local anaesthetic, on a hormone receptor adenylate cyclase system which might be expected to be sensitive to membrane perturbation by local anaesthetics, and reported inhibition of thyro-

tropin (TSH) stimulation of adenylate cyclase in thyroid plasma membranes⁶. We present here a study of the consequence of local anaesthetic activity on binding of the hormone to membrane-associated receptors, and in this respect contrast the local anaesthetic activity of propranolol with that of local anaesthetics such as xylocaine or procaine.

¹²⁵I-TSH binds to plasma membranes derived from the thyroid in a reversible and specific manner, consistent with binding of the hormone to its physiologically relevant receptor linked to adenylate cyclase (see ref. 7 for review). Using experimental conditions previously described⁶, we confirmed that ¹²⁵I-TSH bound to membranes prepared from the human thyroid can be significantly displaced by additions of the order of 25 μ U of unlabelled TSH, and also by immunoglobulins derived from thyrotoxic sera⁹, and have observed that relatively high doses of insulin, growth hormone, luteinising hormone and follicle-stimulating hormone produced no significant inhibition. As shown in Fig. 1, addition of D-propranolol (10^{-3} M) inhibited TSH stimulation of adenylate cyclase in human thyroid membrane preparations, but produced a highly significant increase in binding of the hormone to the membranes. The high concentrations of propranolol required to increase binding (10^{-3} M) and the equipotency of the D and L isomers of propranolol (not shown) together with the observation that quinidine (10^{-3} M) caused a similar increase, suggested that, as was previously reported for the inhibition of adenylate

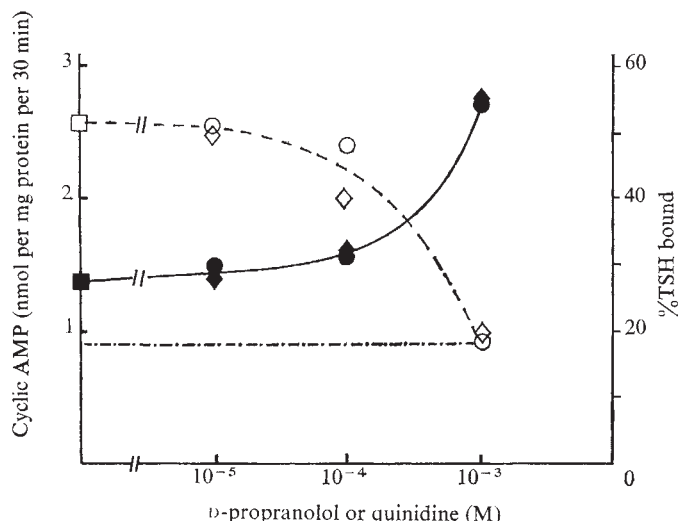


Fig. 1 The effect of propranolol and quinidine on TSH binding and stimulation of adenylate cyclase in human thyroid membranes. Membrane preparation and receptor purification of bovine ¹²⁵I-TSH were as described by Smith and Hall⁶. Binding was studied by incubating the membranes (50 μ l at 2 mg protein per ml) with 200 μ l iodinated bovine TSH (10,000 c.p.m. ml⁻¹) in the presence or absence of propranolol or quinidine, added as 50 μ l of assay buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.1% bovine serum albumin) with or without propranolol or quinidine (quinidine sulphate, Sigma). The incubation was terminated after 1 h at 37 °C by the addition of 1 ml of ice-cold assay buffer, the membranes then being separated by centrifugation (25,000g, 30 min at 4 °C), removal of the supernatant, and bound TSH determined by counting the membrane pellet. Stimulation of adenylate cyclase in the human thyroid membranes by bovine TSH (Armour, 1 U mg⁻¹) was as described before⁶. TSH (100 mU ml⁻¹) was added to membranes (2 mg protein per ml) in the presence of 2.5 mM ATP, 5mM Mg²⁺, 10 mM theophylline, 10 mM creatine phosphate and 20 μ g crystalline rabbit muscle creatine kinase, and the incubation terminated after 30 min at 37 °C. Cyclic adenosine 3',5'-monophosphate was assayed by the method of Broan *et al.*¹⁰. TSH binding (means of duplicate determinations): ■, binding in absence of propranolol or quinidine; ●, binding in the presence of propranolol; ◆, binding in the presence of quinidine. TSH stimulation of adenylate cyclase (means of quadruplicate determinations): - - - - -, basal, unaffected by propranolol or quinidine (10^{-3} M and less); □, TSH stimulated activity in absence of propranolol or quinidine; ○, TSH stimulated activity in presence of propranolol; ◇, TSH stimulated activity in presence of quinidine.

Table 1 The effect of β -adrenergic antagonists on binding of ¹²⁵I-TSH to human thyroid plasma membranes

Additions	% of ¹²⁵ I-TSH bound (less nonspecific)
Experiment 1	
None	28.9 $\pm$ 0.6
D-propranolol (10^{-3} M)	43.7 $\pm$ 1.0
Oxprenolol (10^{-3} M)	32.5 $\pm$ 0.4
Sotalol (10^{-3} M)	30.3 $\pm$ 2.9
Alprenolol (10^{-3} M)	42.2 $\pm$ 0.1
Experiment 2	
None	16.5 $\pm$ 0.8
DL-propranolol (10^{-3} M)	57.4 $\pm$ 2.2
Metoprolol (10^{-3} M)	20.7 $\pm$ 0.9
Experiment 3	
None	22.8 $\pm$ 1.4
D-propranolol (10^{-3} M)	53.5 $\pm$ 0.7
Oxprenolol (10^{-4} M)	22.6 $\pm$ 0.3
(10^{-3} M)	23.2 $\pm$ 1.0
(5×10^{-3} M)	33.9 $\pm$ 0.7
(10^{-2} M)	42.6 $\pm$ 0.2
Practolol (10^{-3} M)	23.2 $\pm$ 0.7
(10^{-4} M)	22.9 $\pm$ 0.01
(10^{-3} M)	21.9 $\pm$ 0.1

Experimental conditions used were as described in legend to Fig. 1. All drugs were in the form of the hydrochlorides, except metoprolol tartrate.

cyclase⁶, the increased binding was attributable to the local anaesthetic properties of propranolol.

We investigated this further by comparing the effects of analogues of propranolol, all of which were β -adrenergic antagonists, but which varied in potency as local anaesthetics. When each drug was added to give a final concentration of 10^{-3} M, increased binding was observed in the presence of alprenolol, which possessed local anaesthetic potency comparable with propranolol and quinidine^{11,12}, but not following the addition of metoprolol, oxprenolol, sotalol or practolol, reported to exert comparatively little local anaesthetic activity^{12,13} (Table 1). But, increasing oxprenolol concentrations to 10^{-2} M resulted in an increased binding which is consistent with its intermediate potency as a local anaesthetic¹².

Table 2 Effect of predicaïne, carbocaine, xylocaine, procaine, and chlorpromazine compared with that of propranolol and quinidine on ¹²⁵I-TSH binding to human thyroid plasma membranes

Additions	% of ¹²⁵ I-TSH bound (less nonspecific)
Experiment 1	
None	22.0 $\pm$ 0.6
D-propranolol (10^{-3} M)	48.4 $\pm$ 0.5
Quinidine sulphate (10^{-3} M)	43.7 $\pm$ 1.2
Predicaïne (10^{-3} M)	22.5 $\pm$ 1.1
(10^{-2} M)	23.7 $\pm$ 0.4
(10^{-1} M)	10.7 $\pm$ 0.6
Carbocaine (10^{-3} M)	22.3 $\pm$ 0.5
(10^{-2} M)	23.3 $\pm$ 0.8
(10^{-1} M)	7.2 $\pm$ 0.2
Xylocaine (10^{-3} M)	22.7 $\pm$ 1.1
(10^{-2} M)	25.0 $\pm$ 0.7
(10^{-1} M)	8.7 $\pm$ 0.5
Experiment 2	
None	22.8 $\pm$ 1.4
D-Propranolol (10^{-3} M)	53.5 $\pm$ 0.7
Procaine (10^{-4} M)	21.1 $\pm$ 0.8
(10^{-3} M)	23.0 $\pm$ 0.2
(10^{-2} M)	20.4 $\pm$ 0.5
(10^{-1} M)	7.3 $\pm$ 0.1
Experiment 3	
None	20.8 $\pm$ 0.5
D-propranolol (10^{-3} M)	37.4 $\pm$ 1.7
Chlorpromazine (10^{-5} M)	21.1 $\pm$ 0.3
(5×10^{-5} M)	20.2 $\pm$ 1.4
(10^{-4} M)	33.1 $\pm$ 0.5
(10^{-3} M)	12.9 $\pm$ 2.2

Experimental conditions were as described in the legend to Fig. 1.

Although increased binding of TSH apparently correlated with the local anaesthetic activity of β -adrenergic antagonists and quinidine, it is interesting that no increase was observed following the addition of the local anaesthetics xylocaine, predacaine, carbocaine and procaine. Detailed investigations, adding concentrations of xylocaine ranging from 10^{-7} to 10^{-1} M caused no increase in binding (not shown) and at concentrations of the order of 10^{-3} M, binding was reduced with xylocaine and the other local anaesthetics (Table 2). Another membrane stabilising agent, chlorpromazine, was found to increase binding when present at 10^{-4} M, although no such effect was reported by Moore and Wolff¹⁴.

Scatchard analysis of the increased binding due to the presence of D-propranolol (10^{-3} M), suggests that it is due to an increase in apparent affinity of the hormone for the receptor, rather than an increase in the number of receptors (Fig. 2). Explaining the nonlinear plots as due to the presence of a high- and low-affinity binding sites, they may be interpreted to indicate an increase in affinity of the high-affinity site from 2.5×10^9 l mol⁻¹ to 8×10^9 l mol⁻¹ in the presence of propranolol.

The detailed mechanism by which propranolol might increase the affinity of the receptor for TSH is unknown, but evidence from several investigations suggests that it may be primarily due to changes in the hydrophobic lipid environment of the membrane-associated receptors. A correlation between increased local anaesthetic potencies of β -adrenergic antagonists and an increase in their hydrophobic nature has been recognised¹², and increased binding of TSH to thyroid membranes has also been reported in the presence of filipin and following membrane treatment with phospholipase A (ref. 14). Moreover, a sharp

increase in binding of TSH observed at temperatures of 30 °C and above has been associated with increased fluidity of membrane lipids, as characterised by motional characteristics of three membrane bound fluorescent probes¹⁵. It is well recognised that TSH stimulation of adenylate cyclase in membranes is sensitive to the presence of phospholipids¹⁶ and the observation that local anaesthetics interact specifically with anionic phospholipids¹⁷ might account for the effect of D-propranolol on the TSH stimulation^{6,18}.

Local anaesthetic activity of β -adrenergic antagonists might also be expected to be effected by local anaesthetics such as xylocaine, and it is significant that despite the correlation between the local-anaesthetic properties of β -adrenergic antagonists and their ability to increase binding, a similar effect was not observed with the local anaesthetics of either the amide or ester type included in this study.

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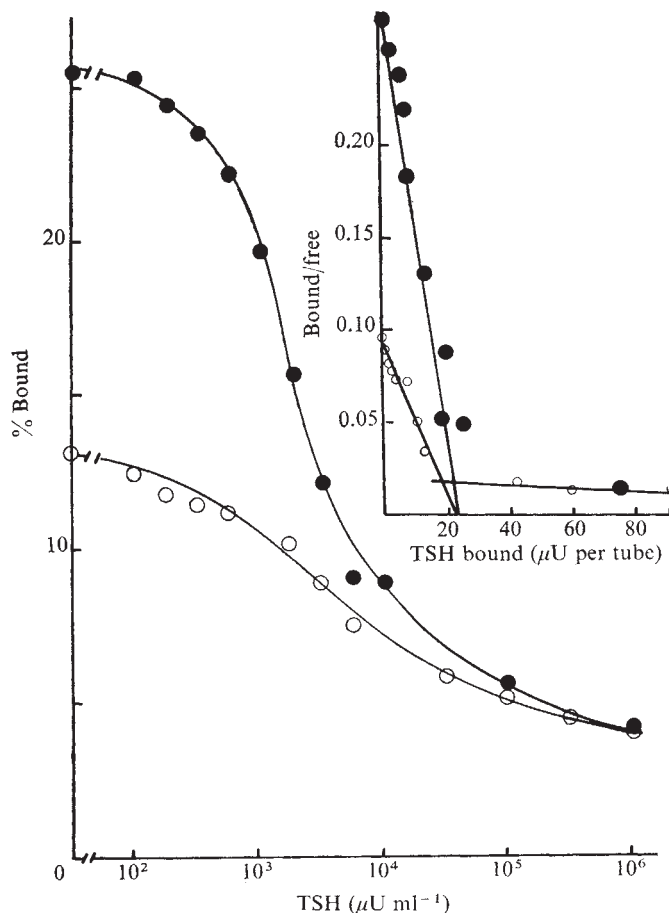


Fig. 2 The effect of propranolol on the binding of bovine ¹²⁵I-TSH to human thyroid membranes in the presence of additional unlabelled bovine TSH. Experimental conditions were as described in the legend to Fig. 1, and unlabelled TSH (Armour, 1 U mg⁻¹) was added at the concentrations shown. The Scatchard plot of these results is shown inset. ○, In the absence of 10^{-3} M D-propranolol; ● in the presence of 10^{-3} M D-propranolol.

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Polypeptides similar to the α and β subunits of tubulin are exposed on the neuronal surface

THE finding by Borisy and Taylor¹ that nervous tissue was the source of large amounts of soluble tubulin, the subunit protein of microtubules, has greatly facilitated the biochemical study of microtubular structure. From 10% to 20% of the total protein of developing brain is in the form of soluble tubulin^{2,3} and most studies have focused on this form of the protein. In 1970, however, Feit and Barondes demonstrated a large amount of colchicine binding activity, a term which has become associated with the presence of tubulin, in particulate fractions of brain⁴—fractions expected to be free of microtubule-derived tubulin. Recent studies indicate that polypeptides with the same size and drug-binding properties as soluble tubulin are present in synaptosomal plasma membranes⁵⁻⁷. These studies suggest a membrane-associated form of the protein which is insoluble in non-chaotropic buffers. I here report experiments which provide additional evidence for membrane-associated

tubulin-like proteins and also suggest that a portion of this polypeptide(s) may be exposed on the external surface of the cell.

Superior cervical ganglia from perinatal rats were separated into three to four pieces and cultured on collagen-coated dishes. The general properties of this nerve cell in culture have been reviewed^{8,9}. These explants were treated with a combination of antimetabolic drugs, in a regimen modified from that used on dorsal root ganglion by Wood and Bunge¹⁰, in order to suppress all cellular growth except that of the neurones. The result of three weeks' growth of four explants in one culture is shown in Fig. 1. The neurones within the explant produce copious neurites, up to 5 mm in length and the dish contains essentially no non-neuronal cells. Such cultures were used to isolate samples of neurites free of their cell bodies and of non-neuronal cells by simple excision and removal of the explant regions from the dish, with subsequent harvest of the extensive neurite outgrowth.

If such explants are given radioactively labelled amino acids in their culture medium, it is possible to demonstrate, by autoradiographic analysis of SDS-polyacrylamide gels of neuronal polypeptides, that the synthesis of tubulin and actin account for as much as one-third of total protein synthesis (ref. 11 and my own data, not shown). Furthermore, since the neurites are metabolically dependent on the cell somata for polypeptides needed in their growth and maintenance, it is possible to study the transport of protein from the somata into the neurites. In pulse-chase experiments of this type, polypeptides co-migrating with tubulin are among the most prominent seen by fluorography (Fig. 2).

When a similar experiment is carried out using fucose as a precursor, polypeptides co-migrating with the tubulin subunits are again heavily labelled (Fig. 3). Comparison of soluble and particulate fractions from these neurites reveals that little or no

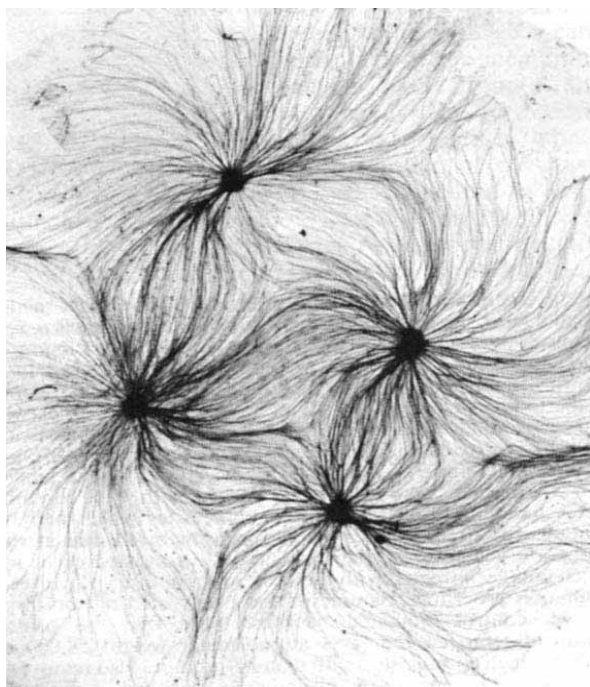


Fig. 1 Photomicrograph of superior cervical ganglion explants after 3 weeks' growth in culture. Cultures were fed with Eagle's minimal essential medium (80 vol. %), supplemented with glucose (600 mg %), human serum (10 vol. %), 150 mM KCl (10 vol. %), and nerve growth factor (20 ng ml⁻¹). The media contained 10⁻⁵ M 5'-fluorodeoxyuridine continuously, and 10⁻⁵ M cytosine arabinoside from day 2 until day 7, *in vitro*. The neuronal cell bodies are confined within the explants and the neurites, free of supporting cells, extend from each explant. Before neurite collection, the explants were removed by dissection. (MX5.)

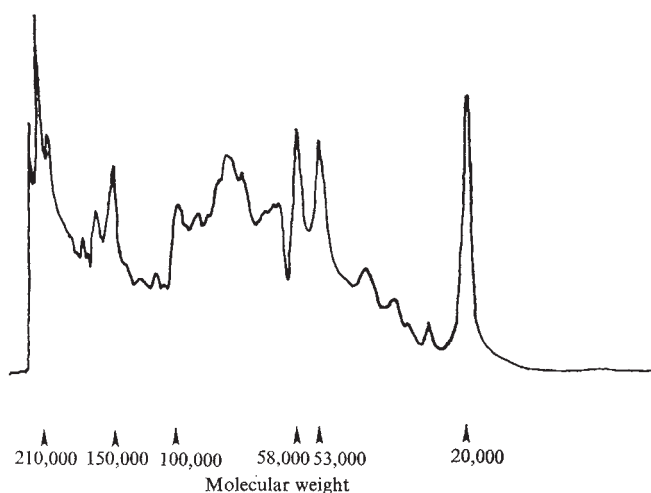


Fig. 2 Densitometric scan of a fluorograph made from contact with an SDS slab gel. The sample was composed of neurites collected from explants which had been exposed to a 2-h pulse of radioactive leucine (20 μ Ci ml⁻¹ in medium made with leucine-free MEM and three times dialysed human serum). The polypeptides were separated by SDS-discontinuous slab gel electrophoresis on a 5–15% gradient of polyacrylamide. These radioactive polypeptides had been transported into the neurites after synthesis in the neuronal somata. The two large peaks of radioactivity at molecular weight 53,000 and 58,000 co-migrate with the α and β subunits of tubulin. The absence of a labelled peak co-migrating with actin is explained by the observation that actin is transported at a slower rate than tubulin and at this moment, has not yet entered the neurite in substantial amounts. Electrophoresis and fluorography were as described in refs 12 and 18. Molecular weight standards and assigned molecular weights were: phosphorylase A (94,000), bovine serum albumin (68,000), catalase (60,000), glutamic dehydrogenase (56,000), IgG, heavy chain (50,000), ovalbumin (43,000), aldolase (40,000), lactate dehydrogenase (36,000), α -chymotrypsinogen (25,000), IgG, light chain (23,500), myoglobin (17,200). Porcine brain tubulin was used as standards for α and β tubulin.

fucose-derived radioactivity is associated with the soluble tubulin but that the particulate fraction contains heavily labelled polypeptides co-migrating with tubulin.

This experiment suggested that polypeptides similar to the α and β subunits of soluble tubulin were associated with the neuritic plasma membrane. In order to investigate the polypeptides of the plasma membrane, external labelling using iodination catalysed by glucose oxidase/lactoperoxidase was attempted. The results of such experiments are shown in Fig. 4. A relatively small number of polypeptides became labelled in conditions in which the enzymes should have remained outside the neurites (Fig. 4a, c). That the labelling pattern is due to iodination of polypeptides exposed on the cell exterior is supported by a number of control experiments: (1) the labelling is entirely dependent on the presence of the catalytic enzymes; (2) the labelling pattern which homogenised neurites exhibit is much more inclusive, with essentially all of the polypeptides demonstrated by Coomassie blue staining labelled. The most striking example of this is the heavy labelling of polypeptides co-migrating with actin, when the iodination is done on homogenised neurites as compared to the absence of label in that region after iodination of intact neurites (experiment not shown); (3) none of the media polypeptides or substrate polypeptides show an iodination pattern similar to that in Fig. 4, when examined electrophoretically. This latter control suggests that the surface-associated tubulin-like proteins are not derived from polypeptides present in the medium.

The effects of trypsin treatment on the iodination pattern further support the hypothesis that the tubulin-like polypeptides are exposed at the exterior surface of the neuronal plasma membrane. Figures 4d and e show that brief treatment with low levels of trypsin will remove the iodlatable species which co-migrate with soluble reference tubulin. This suggests a

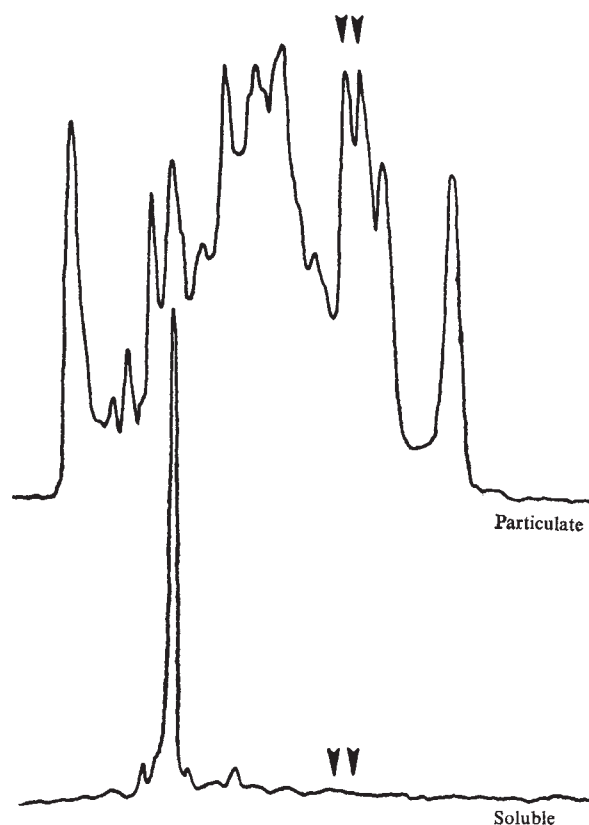


Fig. 3 Densitometric scans of fluorographs made from contact with SDS-slab gels. The samples were derived from neurites harvested from explants after 12 h of labelling with tritiated fucose ($10 \mu\text{Ci ml}^{-1}$ in medium made with three times dialysed serum). In the upper scan, the sample consisted of insoluble neuritic material after homogenisation in 150 mM NaCl, 5 mM sodium phosphate (pH 7.2), and centrifugation at $100,000g$ for 1 h. The lower scan represents the soluble neuritic material derived from the same homogenate. In the lower scan, there is no radioactivity associated with the area of the gel containing the α and β subunits of tubulin (arrows). In the particulate sample, however, polypeptides co-migrating with α and β tubulin subunits are substantially labelled. Direction of electrophoresis was from left to right. Electrophoresis and fluorography as in Fig. 2.

restricted amount of the iodinated species, most or all of which is removed in the proteolytic treatment. Data not shown demonstrate that there are no differences in the Coomassie blue staining patterns of the samples used in Fig. 4c, d, e indicating the small amount of proteolysis which actually occurs during such treatment, and more importantly, that the bulk of the neuritic tubulin is unaffected while the co-migrating label is removed.

In the Laemmli SDS gel system¹², the α and β subunits of soluble tubulin are resolved and migrate at molecular weight 58,000 and 53,000, respectively. If the ionic strength of this same buffer system is increased by the addition of 300 mM NaCl, the tubulin subunits co-migrate¹³. When aliquots of the same sample of iodinated neurites are run in the two gel systems, the labelled polypeptides in the molecular weight range 50,000–60,000 always co-migrate with soluble reference tubulin subunits no matter whether the reference tubulin subunits resolve (as in the regular Laemmli system) or not (as in the high ionic strength modification). Thus, in two quite different gel systems, these two polypeptides behave similarly to soluble tubulin.

Taken together, these experiments strongly suggest and strengthen the view that tubulin-like polypeptides are present in the neuronal plasma membrane and further, that these polypeptides may be exposed on the exterior of the cell surface.

The role of such a protein may only be hypothesised at this time. It is well known that a colchicine-sensitive mechanism

can control the mobility of polypeptides in the cell membrane¹⁴. These membrane-associated tubulin-like polypeptides may act as anchors for the membrane-associated microtubules described by a number of electron microscopists. In terms of neuronal cell biology, Levi-Montalcini *et al.* have presented evidence that a tubulin-like molecule may have a role in the recognition of nerve growth factor (NGF) at the cell surface¹⁵. In their study, live intact mouse neuroblastoma cells, which had NGF receptors on their surfaces, would form rosettes with

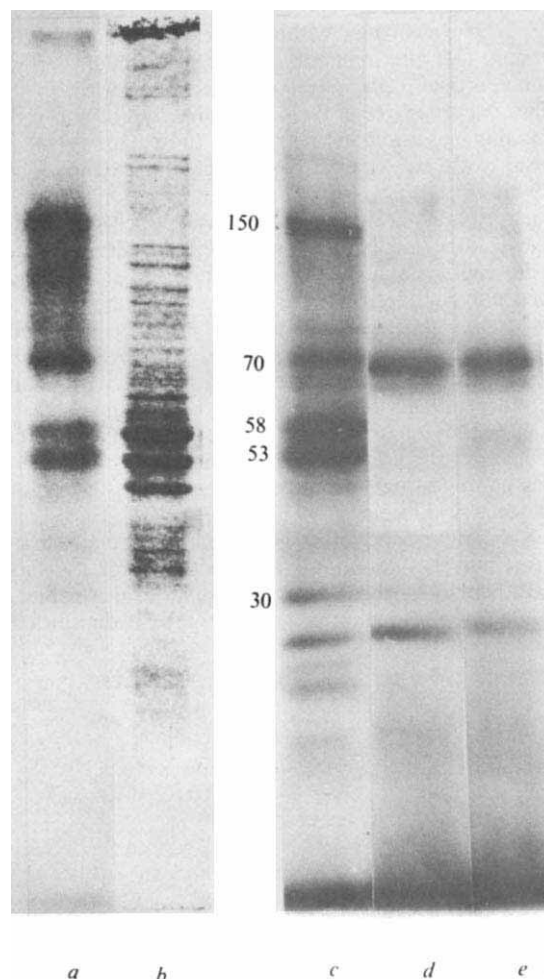


Fig. 4 Results of iodination experiments on intact neurites isolated from superior cervical ganglion explants. Panels a, c, d, e are autoradiographs of SDS-slab gels. Panel b is a picture of a Coomassie blue stained gel which produced the autoradiograph shown in panel a, and allows for molecular weight assignments of the iodinated bands. All samples consisted of neurites which had been iodinated using 5 milliunits ml^{-1} each of lactoperoxidase and glucose oxidase, 5 mM glucose, and 100 mCi ml^{-1} Na^{125}I , in phosphate-buffered saline, pH 7.2. After 15 min labelling at 0°C , samples were repeatedly washed with PBS containing excess iodide. Sample d consisted of neurites which had been treated with $20 \mu\text{g ml}^{-1}$ crystalline trypsin for 15 min at room temperature before iodination. Sample e consisted of neurites which had been treated with the same amount of trypsin after the iodination procedure. Samples a and c consisted of non-trypsin treated, control iodinated neurites. Note that the protease treatment removed the label at molecular weight 53,000 and 58,000, which co-migrated with α and β tubulin. Also removed by protease treatment were prominently labelled polypeptides migrating at molecular weight 150,000 and 30,000. In sample B, the prominent polypeptide band below the heavy bands at molecular weight 53,000 and 58,000 co-migrated with purified muscle actin. Note the absence of any iodine incorporation into this major polypeptide in the autoradiographs of the iodinated neurites. The numbers in the centre of the figure indicate approximate molecular weight $\times 10^{-3}$, as determined by comparison with polypeptides of known molecular weights. Electrophoresis was as described in ref. 12. Autoradiography was accomplished by exposing Kodak RPR-54 film to the stained and dried slab gel.

NGF-coated red cells. This rosette formation presumably represented the hormone-receptor interaction. Antibody prepared against soluble brain tubulin could prevent rosette formation in their experiments.

Actin and myosin have now been shown to be associated with the surface membrane in a variety of non-muscle cells^{16,17}. The identification of tubulin in the surface membrane of neurones suggests that it will be necessary to expand our ideas of the roles this protein may play in cell function.

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Type I and type II cyclic AMP-dependent protein kinase as opposite effectors of lymphocyte mitogenesis

THE role of cyclic AMP in the regulation of cell growth and differentiation has been extensively investigated in many cell types following various growth stimuli^{1,2}. In recent years, peripheral blood lymphocytes treated with antigens³, plant lectins⁴, lipopolysaccharides⁵, or periodate oxidation⁶ have served as a model system for assessing whether cyclic AMP is involved in the regulation of the mitogenic response promoted in these cells by the above classes of agents^{7,8}. There are two conflicting hypotheses, as outlined by Friedman¹, concerning the role of cyclic AMP in lymphocyte proliferation. The first hypothesis suggests that the increase in cyclic AMP levels following mitogen stimulation⁸ and the subsequent increase in ³²P incorporated into FI histones⁹ and certain cytosol proteins¹⁰ implicates cyclic AMP as a positive mediator of mitogenesis in lymphocytes. The other hypothesis reasons that cyclic AMP inhibits proliferation, and this hypothesis is supported primarily by observations from several laboratories that raising the intracellular cyclic AMP level through the use of phosphodiesterase inhibitors¹¹, prostaglandins¹², or cyclic AMP analogues^{11,13-15} effectively inhibits mitogen-stimulated RNA and DNA synthesis. We present here evidence that may help resolve this apparent paradox. Incubation of Ficoll-Hypaque purified human peripheral blood lymphocytes with concanavalin A (conA) leads to the activation of only type I cyclic AMP-dependent protein kinase in these cells even though there are significant amounts of both type I and type II kinase present. But, the addition of dibutyryl cyclic AMP (DBcAMP) to the conA-stimulated lympho-

cytes at a concentration sufficient to block the synthesis of RNA and DNA results in the activation of both type I and type II cyclic AMP-dependent protein kinase. It therefore seems likely that while the activation of type I protein kinase represents a positive component in the progression of events promoted in a lymphocyte by a mitogenic signal (as it does in other cell types in which a trophic response is evoked), the activation of type II protein kinase (or perhaps types I and II in concert) represents a mechanism by which a negative influence can be imposed on the pro-

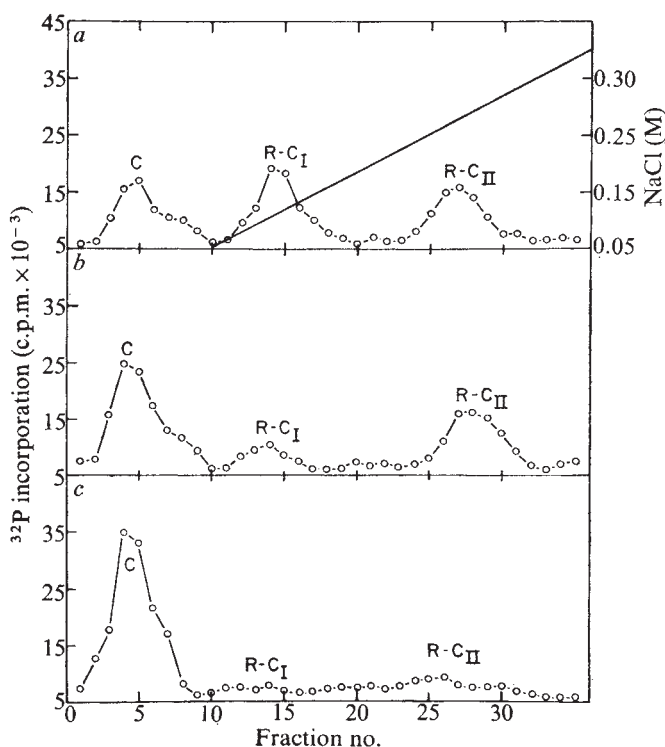


Fig. 1 Type I and type II protein kinase activation following con A and DBcAMP administration. Human peripheral blood lymphocytes were isolated from heparinized blood from healthy male donors by flotation on Ficoll-Hypaque (Lifton Bionetics) and washed three times using phosphate buffered saline as described previously²³. The cells were suspended in 10 ml of Eagles medium at a density of 2×10^6 per ml and pre-incubated at 35 °C overnight. Con A was added at $10 \mu\text{g ml}^{-1}$ resulting in maximal rates of RNA and DNA synthesis at 48 and 72 h respectively. Previous experiments using supernatant determinations of the without cyclic AMP/with cyclic AMP protein kinase activity ratio (data not shown) indicated that total cyclic AMP-dependent protein kinase activation was greatest after a 4-h incubation with con A. DBcAMP 10^{-5} M when added concomitant with con A blocked by greater than 90% the increase in ³H-uridine and ³H-thymidine incorporation into acid-precipitable material normally seen at 48 and 72 h. C⁶-amino alkyl Agarose chromatography of unstimulated (a), con A (b), and con A and DBcAMP (c) stimulated lymphocytes (4-h incubations) was performed as described by Rangel-Aldao and Rosen¹⁶ with modifications. The C⁶-Agarose was prepared according to Shaltiel²⁵ and 1-ml columns of resin were equilibrated with 15 mM sodium-potassium phosphate, pH 6.8, 1 mM EDTA, 0.5 mM methylisobutylxanthine, 5 mM β -mercaptoethanol, 5 mM NaFl, and 60 mM NaCl. This salt concentration was sufficient to allow the free catalytic subunit to pass through the column while the regulatory subunits and type I and type II holoenzyme were bound. The capacity and the required initial salt concentration was determined with purified C, R₁C (type I), and R₁₁C (type II) for each batch of resin. 60×10^6 cells were sonicated for 10 s in 300 μl of the above buffer at 0 °C and 250 μl of the sonicate applied to the column. The column was rapidly washed for 10 fractions (200 μl per fraction) with buffer containing 60 mM NaCl and a gradient from 60 to 350 mM NaCl (4 ml of each) begun. Aliquots (50 μl) of each fraction were assayed for kinase activity with mixed calf thymus histone as substrate in the presence of 5 μM cyclic AMP (ref. 19). Purified regulatory and catalytic subunits showed no reassociation when chromatographed in these conditions.

liferative process by the generation of abnormally high levels of intracellular cyclic AMP.

Human peripheral blood lymphocytes were purified and cultured as described in the legend to Fig. 1. Using C⁶-amino alkyl Agarose chromatography, originally used by Rosen and coworkers to study isozymes of cyclic AMP-dependent protein kinase^{16,17}, the two isozyme forms (R_IC and R_{II}C, inactive holoenzyme forms) were rapidly separated from each other and from the dissociated free catalytic subunit (C), that is, activated protein kinase. Con A addition, which promotes an increase in cyclic AMP (ref. 8), activates only type I of the kinase. Fig. 1a, b shows that kinase activity decreases in those fractions containing type I holoenzyme and appears in the flow-through fractions which correspond to free active catalytic subunit. Addition of DBcAMP to the con A-stimulated lymphocytes further reduces kinase activity from the elution profile which corresponds to type II holoenzyme and again the activity is recovered as catalytic subunit in the flow-through fractions (Fig. 1c).

The mechanism by which the activation of type II kinase by DBcAMP in lymphocytes might lead to inhibition of mitogen-induced proliferation is not immediately apparent. However, the activation of both forms of the kinase rather than the selective activation of type I at a time when the cell is preparing to synthesise protein, RNA, and DNA in a specific series of events leading to eventual cell division may interfere with any of a multitude of parameters mediating this process. These could include Ca²⁺ fluxes, ribosome aggregation, cyclic GMP events, and/or transport of precursors or essential serum factors from the media.

It is becoming increasingly evident that type I and type II cyclic AMP-dependent protein kinase may have separate functions in the cell. They have specific expression patterns during cell cycle¹⁸, cardiac hypertrophy¹⁹, and during the growth and differentiation of certain tissues²⁰. Lee *et al.*²⁰ have shown that during testicular ontogeny, type I protein kinase is present and the appearance of differentiated cell types in the testis is concomitant with an increase in type II protein kinase. In addition, the two forms of protein kinase have different regulatory properties. Rosen¹⁶ has shown that only the regulatory subunit of type II holoenzyme is phosphorylated, greatly reducing its ability to reassociate with free catalytic subunit. A separate role was postulated for this isozyme based on this unique phosphorylation event. In those cell culture cells in which cyclic AMP events have been studied, cyclic AMP increases markedly during G₁ phase of the cell cycle, decreases at the G₁/S border, and increases again in G₂/M. Addition of DBcAMP and a phosphodiesterase inhibitor early in G₁ prevents Chinese hamster ovary cells from progressing from G₁ into S phase²¹. In fact, it also results in differentiation as assessed by cell elongation and increased microtubular structures^{21,22}.

Therefore, while activation of type I cyclic AMP-dependent protein kinase represents a positive component in events promoted in the lymphocytes by a mitogenic signal, the activation of type II protein kinase (or perhaps types I and II in concert) represents a mechanism by which a negative influence can be imposed on the proliferative response by the generation of an abnormally high intracellular cyclic AMP concentration. This study for the first time points to the complexity of cyclic AMP mediation, since it acts through two specific enzymes regulated by alterations in cyclic AMP levels.

Further studies of tissues at various stages of differentiation in regard to pools of protein kinase, type I and type II, as well as the activation patterns of these kinases after intracellular changes in cyclic AMP in response to hormones which are specific stimuli and to the addition of cyclic nucleotide analogues and phosphodiesterases

which are nonspecific stimuli are necessary to ascertain if these parameters operate in the control of growth and differentiation.

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Chinese hamster cell mutant with defective mitochondrial protein synthesis

STABLE mutations affecting mitochondrial functions have been reported in lower eukaryotes¹⁻⁷ and higher organisms⁸⁻¹¹. We have described a procedure to select mutants with defective patterns of oxidative energy metabolism from established Chinese hamster fibroblasts⁸, which seem to satisfy most of their energy needs from glycolysis⁶. We describe here a mutant which has properties reminiscent of the petite phenotype of yeast ρ^- mutants^{3,13,14}: a defect in mitochondrial protein synthesis leads to multiple enzymatic deficiencies.

The mutant cells, V79-G7, were characterised as respiration-deficient by the following criteria⁸⁻¹⁰. (1) The rate of oxygen consumption by whole cells is about 10% that of wild-type cells. (2) There is no appreciable release of ¹⁴CO₂ from 2-¹⁴C-pyruvate or 5-¹⁴C-glutamate (conversions requiring an active and complete Krebs cycle). (3) The cells behave as auxotrophs for CO₂ and asparagine, compounds normally derived from the Krebs cycle. And (4), when galactose, a slowly fermentable carbon source, is substituted for glucose in the medium, the cells die overnight. In these respects V79-G7 cells resemble all other respiration-deficient fibroblasts isolated from the parental line⁸. A unique property of this mutant was a reduced capacity of isolated mitochondria to respire in the presence of succinate or α -glycerolphosphate. Succinate dehydrogenase activity in mitochondria was much less reduced (Table 1). These results focused our attention on the electron transport chain between coenzyme Q and oxygen.

The terminal enzyme complex of the electron transport chain is cytochrome oxidase which can be assayed in purified mitochondria using exogenous, heterologous, reduced cytochrome *c* as substrate¹⁵. In Fig. 1 a rate constant,

Table 1 Biochemical activity of V79 and V79-G7 mitochondria

	Activity per mg protein		% of control
	V79	V79-G7	
Succinate oxidation	1180 ± 120 nmol O ₂ h ⁻¹	400 ± 90 nmol O ₂ h ⁻¹	33.9 ± 7.6
α-glycerol phosphate oxidation	1320 ± 190 nmol O ₂ h ⁻¹	700 ± 40 nmol O ₂ h ⁻¹	53.0 ± 3.0
Cytochrome oxidase activity	24.4 ± 12.3 ml min ⁻¹	1.6 × 0.8 ml min ⁻¹	6.6 ± 3.3
ATPase activity (oligomycin-sensitive)	380 ± 56 nmol P _i h ⁻¹	44 ± 11 nmol P _i h ⁻¹	11.6 ± 2.9
Succinate dehydrogenase	23.7 nmol mg ⁻¹	17.0 nmol min ⁻¹	72

Assays of selected activities of isolated mitochondria. Cells were grown in Dulbecco's modified Eagle's medium (DME) with the following additions: 45 µg ml⁻¹ asparagine, 4 mg ml⁻¹ glucose, 20 mM HEPES buffer, pH 7.5, 10% foetal calf serum (Irvine Scientific) and alternating antibiotics. Mitochondria were isolated following the procedure of White and Tewari²⁵. Washed cells were homogenised in a Dounce homogeniser in buffer containing 250 mM sucrose, 2 mM EDTA, 50 mM Tris, pH 7.4 (SM buffer), containing 1% bovine serum albumin (BSA). After removal of the nuclei the mitochondria were pelleted in the SS34 Sorvall rotor (10,000 r.p.m., 10 min) washed once by resuspension in SM buffer plus 1% BSA and repelleted as above. All manipulations were carried out at 0–4 °C. For the measurement of oxygen consumption mitochondria were resuspended in 2 ml SM buffer plus 1% BSA, placed in a thermostated oxygen electrode chamber, and oxygen consumption was measured as described previously⁹. The cytochrome oxidase assay is described in the legend to Fig. 1. The oligomycin-sensitive ATPase was found to be most reproducibly measured after treatment of the mitochondria at pH 9.2, in SM buffer, at room temperature²⁶. 100 µl of mitochondrial suspension was then mixed with 350 µl of Tris buffer (25 mM, pH 9.2) containing Mg²⁺ (3.3 mM), plus or minus oligomycin (44.4 µg ml⁻¹), and finally 50 µl of ATP (50 mM) was added to the mixture. After 10 min at 30 °C the reaction was terminated by addition of trichloroacetic acid (final concentration 10%) and inorganic phosphate determined by the method of Fiske and Subbarow²⁷. Approximately 60% of the total ATPase activity observed in wild-type mitochondria was inhibitable by oligomycin. Mg²⁺ ions were essential for all observed activities.

assumed to be proportional to cytochrome oxidase activity is plotted against amounts of Lubrol added to the mitochondrial preparation. Treatment of wild-type mitochondria with detergent uncovered measurable activity, whereas in the mutant, enzyme activity remained very low, at most 6.6% relative to wild type.

These results were confirmed by measurements of the absorbance spectra of fully reduced whole cells in the wavelength range 400–630 nm (Fig. 2). The most notable differences between mutant and wild-type cells were observed at 440 nm and at 600 nm corresponding to the Soret band and the α band, respectively, of the cytochromes *aa*₃. Cytochromes *aa*₃ seem to be absent in the mutant cells. Although uncertainties of the baseline make quantitative comparisons difficult, there seems to be no decrease in the cytochrome *c* content of the mutant cells, as judged by the absorption peak at 550 nm. But, the shoulder at 560 nm, the region of absorption of cytochrome *b*, seems to be reduced in the case of the mutant cells.

From work done in yeast and *Neurospora*, it is known that the cytochrome oxidase and cytochrome *b* contain subunits which are synthesised inside the mitochondria, whereas cytochrome *c* is synthesised exclusively in the cytoplasm^{13,14,16,17}. Another enzyme known to have several subunits synthesised within the mitochondria is the oligomycin-sensitive ATPase (refs 13, 14, 18). We assayed this enzyme in mitochondria and found that its activity, like that of cytochrome oxidase, was greatly reduced in the mutant cells (11.6% relative to wild type, see Table 1).

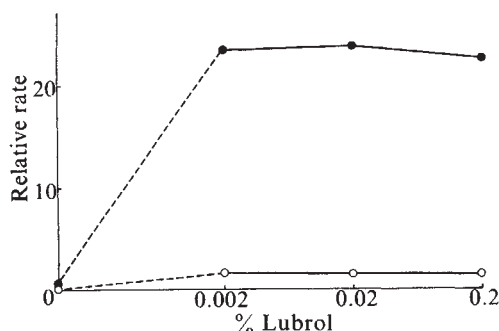


Fig. 1 Cytochrome oxidase activity in detergent-treated wild-type and mutant mitochondria. After a 10-min pretreatment of the mitochondria in SM buffer with various concentrations of Lubrol the reactions were initiated by addition of excess reduced cytochrome *c* from beef heart¹² and the absorbance was measured at 550 nm with a recording spectrophotometer. Experiments were performed at 25 °C. The observed rate constants were normalised to the same concentration of total mitochondrial protein in the reaction mixture. Absolute rates are given in Table 1.

The properties of the mutant described so far, and the similarity to the phenotype of the ρ^- mutants of yeast, prompted us to examine mitochondrial protein synthesis *in vivo* in wild-type and V79-G7 cells. In these experiments cytoplasmic protein synthesis was inhibited by cycloheximide, and the cells were then labelled with ³H-leucine, followed by a chase with cold leucine. In wild-type cells cycloheximide, at 100 µg ml⁻¹, inhibited total incorporation of ³H-leucine into whole cells by 95–97%. Purified mitochondria were dissolved in buffer containing SDS and β-mercaptoethanol, and their proteins were fractionated electrophoretically on sodium dodecyl sulphate–polyacrylamide slab gels (Fig. 3). The wild-type pattern shows at least seven peaks of radioactivity, corresponding to a range in size from 10,000 to 44,000 MW (Fig. 3a). In contrast, an equivalent amount of mitochondrial protein from the mutant cells gave no such distinctive profile (Fig. 3b). Only traces of the 22,500 and 25,000 peaks were visible. The amount of ³H-leucine incorporated per milligram of protein of the mitochondrial fraction was slightly greater than one-third of that in wild-type cells. Most of the radioactivity

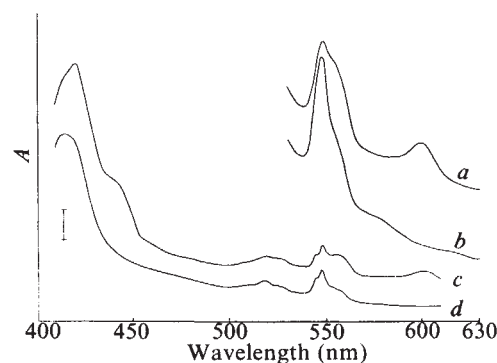


Fig. 2 Absorption spectra of reduced, whole cells. A suspension of cells in buffer containing NaCl (137 mM), KCl (5 mM), Na₂HPO₄ (0.7 mM), Tris (25 mM), pH 7.3, was reduced by addition of a few grains of dithionite and then frozen in quartz spectrophotometer cuvettes at a density of 10⁷ cells per ml. Absorption spectra were recorded at liquid nitrogen temperature in a Cary 14 spectrophotometer, with the output from the photomultiplier fed into a PDP 8/1 computer for the summation of multiple scans over the wavelength region indicated. Two independent experiments are shown: wild-type (c) and mutant cells (d) were scanned from 410 to 610 nm, and the experiment was repeated with different batches of cells: wild-type (a) and mutant cells (b), with a scan from 530 to 630 nm. Approximately equal numbers of wild-type and mutant cells were compared in each experiment. The bar represents an absorbance change of 0.1 (curves c and d), and the scale is changed by a factor 4 for curves a and b.

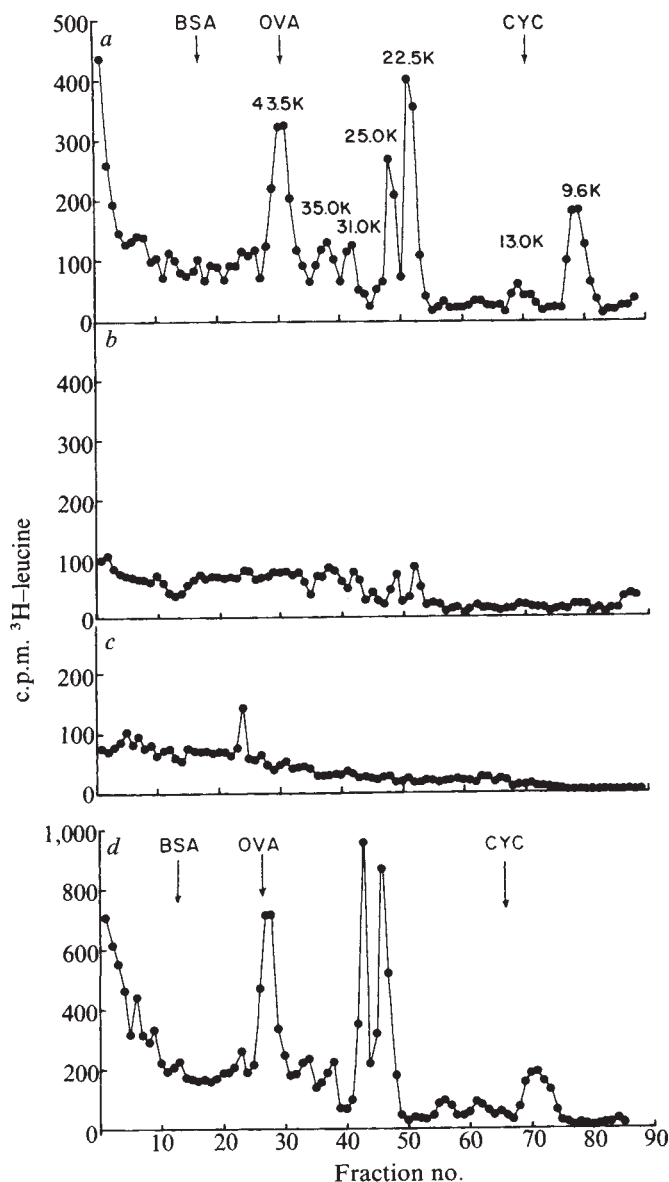


Fig. 3 Protein synthesis in wild-type and mutant mitochondria. Cells grown in roller bottles ($\sim 5\text{--}7 \times 10^7$ cells total) were incubated for 30 min with $100 \mu\text{g ml}^{-1}$ cycloheximide, and then labelled for 2 h with ^3H -leucine ($20 \mu\text{Ci ml}^{-1}$; specific activity $57.4 \text{ Ci mmol}^{-1}$), followed by a 30-min chase with an excess of cold leucine ($100 \mu\text{g ml}^{-1}$). The mitochondria were purified first as described in the legend of Table 1, then loaded on top of linear sucrose gradients ($1.03\text{--}1.60 \text{ M}$ sucrose, 50 mM Tris, $\text{pH } 7.4$, 2 mM EDTA) and centrifuged at 4°C in a Beckman SW50.1 rotor at $40,000 \text{ r.p.m.}$ for 2 h. A narrow band of material was always observed at a density of 1.17 g cm^{-3} , and a peak of radioactivity was associated with this band. The band was removed, the material pelleted by centrifugation, dissolved at $90\text{--}100^\circ\text{C}$ in buffer containing Tris-HCl (62.5 mM , $\text{pH } 6.8$), glycerol (10% w/v), β -mercaptoethanol (5% w/v), sodium dodecyl sulphate (SDS, 2.3% w/v), and stored at -20°C until ready for use in electrophoresis. Samples were run on 12.5% polyacrylamide slab gels (1 mm thickness) containing Tris-HCl (37.5 mM , $\text{pH } 8.8$) and SDS (0.1% w/v). The gels were stained with 0.1% Coomassie blue in 50% trichloroacetic acid for 20 min, destained in 7% acetic acid, and then cut into 1-mm sections with a gel slicer made of a battery of razor blades. Slices were incubated overnight in 0.5 ml NCS solubiliser (Amersham/Searle) before being counted in 5 ml of toluene-based scintillation fluid. Bovine serum albumin (BSA), ovalbumin (OVA) and cytochrome *c* (CYC) were run on the same gels in parallel tracks as molecular weight markers. Profiles for mitochondria from: *a*, V79 wild type; *b*, V79-G7; *c*, V79 pretreated with chloramphenicol at $100 \mu\text{g ml}^{-1}$ for 5 h before addition of label; *d*, CCL16-B2, a mutant lacking NADH-oxidase activity. Radioactivity content: *a*, $469 \text{ c.p.m. } \mu\text{g}^{-1}$; *b*, $196 \text{ c.p.m. } \mu\text{g}^{-1}$; *c*, $158 \text{ c.p.m. } \mu\text{g}^{-1}$; *d*, $836 \text{ c.p.m. } \mu\text{g}^{-1}$.

was distributed as a background or relatively high molecular weight material.

To prove that the observed pattern of protein synthesis is indeed due to mitochondrial protein synthesis, we tested its sensitivity to the specific inhibitor chloramphenicol (Fig. 3c). The effect of this inhibitor is very marked, although we found it necessary to pre-incubate the cells for some time with the drug before the addition of ^3H -leucine. With wild-type V79 cells we found approximately 64% inhibition of cycloheximide-resistant ^3H -leucine incorporation as well as an altered profile (Fig. 3c). Pretreatments longer than 5 h failed to reduce the level of incorporation further. We tentatively conclude that there is a background of chloramphenicol-resistant protein synthesis in this system (which may in fact be due to incomplete inhibition of cytoplasmic synthesis) which accounts for approximately one-third of the incorporation seen in the absence of the drug.

The abnormal pattern of mitochondrial protein synthesis in V79-G7 cells is not a general characteristic of respiration-deficient Chinese hamster fibroblasts. Figure 3d shows a profile corresponding to mitochondrial protein synthesis in a mutant defective in NADH-coenzyme Q reductase^{9,10}. Except for a difference in specific activity there is no significant difference between this pattern and that observed for wild-type cells, and similar results were observed with yet another respiration-deficient mutant representing a third complementation group (results not shown). We conclude that the defect in mitochondrial protein synthesis in V79-G7 cells is unique.

We do not yet know whether the mutation in the V79-G7 cells is nuclear or cytoplasmic. In fact, this mutant is very similar in phenotype to a nuclear yeast mutant (strain M126) described by Colson, Labaille and Goffeau¹⁹. Mitochondrial DNA is known to code for a limited number of macromolecules including ribosomal RNAs, transfer RNAs and probably certain subunits of cytochrome oxidase, cytochrome *b*, and the oligomycin-sensitive ATPase (refs 13, 14, 16, 17, 20), while most if not all of the ribosomal proteins, RNA polymerase, aminoacyl-tRNA synthetases, and other protein factors involved in protein synthesis are thought to be coded for by nuclear genes and imported from the cytoplasm^{21,24}.

Studies are in progress aimed at (1) the fusion of enucleated, cytoplasmically marked (chloramphenicol-resistant) hamster cells with the V79-G7 cells; (2) measurement of the spontaneous reversion rate; and (3), physical characterisation of the mitochondrial DNA from the mutant. Further investigation of this mutant may allow further comparison of mitochondrial biogenesis in eukaryotic cells such as yeast and in mammalian cells, and of the contributions which the nuclear and mitochondrial genomes make to the assembly of the organelle.

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Interferon production during lymphocytic choriomeningitis virus infection of nude and normal mice

SEVERAL reports¹⁻⁶ have indicated that lymphocytic choriomeningitis virus (LCMV) infection of mice or cultured cells is not associated with interferon production. But, as was found later⁷ the methods used may not have been sensitive enough to detect interferon in tissues of virus infected animals. In contrast to these earlier observations, mice acutely infected with M-P virus, a virus closely related to LCMV (ref. 8), have been reported to produce interferon⁹. We observed a more intense and prolonged interferon response to a virus inducer in nude athymic mice (Fig. 1) as others¹⁰ have with synthetic double-stranded nucleic acids. Therefore, it seemed reasonable to study LCMV infection of nude animals carefully for interferon production in serum and infected organs. Parental strains and nude heterozygotes (Nu/Nu^+) were studied as well as the homozygous nude (Nu/Nu) animals. The results presented here indicate that interferon is made in greater quantity and persists longer during infection of Nu/Nu than of parental and Nu/Nu^+ mice.

Figure 1 presents results following intravenous inoculation of live Newcastle disease virus in Nu/Nu , Nu/Nu^+ and BALB/c parental strains, as well as similar parental and nude mice of a CBA background. Results from two separate serum pools involving 10 animals each were averaged for both comparative studies. It can be seen that the parental responses are consistently lower than those of nude animals at all time points, particularly at 12 and 24 h after virus inoculation. The Nu/Nu^+ mouse in our studies with the CBA strain resembles the nude mouse but is intermediate in its responses in the BALB/c animals. These findings resemble those reported by Yokota, *et al.*¹⁰ with poly I : C injection of BALB/c, nude and heterozygote animals, and indicate that interferon production can occur in the absence of thymus dependent lymphocytes.

Studies with acute infection demonstrated high interferon titres in the spleen and serum in all 6-week-old animals studied 4-7 d after intracerebral (i.c.) inoculation with 100 LD₅₀ of LCMV, Armstrong strain (Table 1). This time period corresponds to the period of maximal T-cell activity and other immunopathological responses. In the nude animals there was an increase in spleen and serum levels of interferon, four- and threefold respectively on the seventh day after infection compared with the fourth day after infection. In contrast, at least an eightfold reduction in interferon levels was observed in the spleens and serum from Nu/Nu^+ and parental BALB/c animals studied at

similar times (Table 1). The antiviral material extracted from the spleen and serum was shown to be interferon by its stability to pH 2 for 1 h at 4 °C, lack of sedimentability at 100,000g for 2 h, its inactivation by trypsin (1 mg ml⁻¹, 37 °C 1 h), its immunospecific neutralisation on exposure to antisera to L cell interferon¹² and its lack of protection of human skin fibroblasts challenged with vesicular stomatitis virus. Furthermore, no protection was observed when the antiviral substance was applied to L cells for only a short period (1 h) before virus challenge even

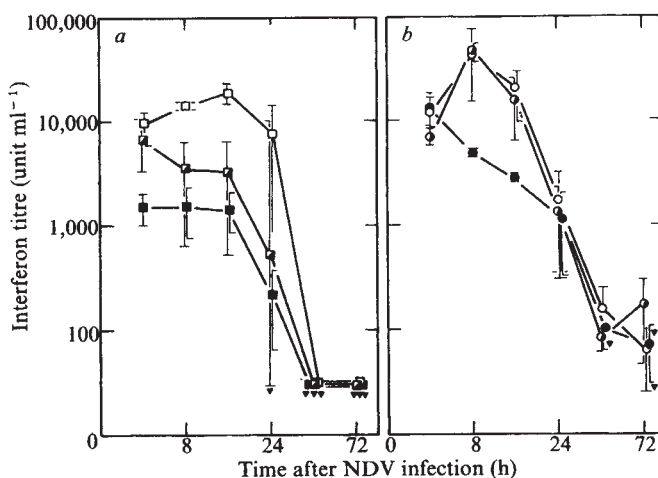


Fig. 1 Production of interferon in Nu/Nu , Nu/Nu^+ and BALB/c or CBA parental strains. *a*, BALB/c, Nu/Nu and Nu/Nu^+ ; *b*, CBA, Nu/Nu and Nu/Nu^+ . Each point represents the mean of two experiments in which serum from 10 animals was pooled for each experimental observation. The serum was collected at the indicated times after inoculation of 20-g mice with 1×10^8 plaque-forming units of live Newcastle disease virus (NDV) intravenously. Interferon titres represent the reciprocal of the dilution of serum giving 50% inhibition of vesicular stomatitis virus plaque formation in the L₉₂₉ monolayers growing in 60-mm plastic plates as described elsewhere¹¹. The mean and range of replicates is shown by the bracketed bar. Symbols: in *a*—■, homozygote BALB/c parent (BALB/c); ▣, BALB/c nude heterozygote (Nu/Nu^+); □, nude homozygote (Nu/Nu). In *b*, ●, CBA parent (CBA); ⊙, CBA nude heterozygote (Nu/Nu^+); ○, nude homozygote (Nu/Nu). In all strains, interferon titres in the serum at zero time were less than 20 units per ml.

though it was maintained on monolayers during plaque development.

Interferon levels in the liver and kidney were similar in all animals 4 d after LCMV inoculation, while at the seventh day post-infection, nude mice showed higher levels of interferon when compared to Nu/Nu^+ and parental mice. Despite high virus titres and in contrast to certain other murine viral encephalidites, interferon titres in the brain were low and indistinguishable in all three types of animal. It is of interest that there were higher virus titres in spleen, liver and kidney tissues of nude mice 7 d after infection as compared with Nu/Nu^+ and BALB/c animals. The nude animals also survived acute LCMV infection in contrast to the parental and Nu/Nu^+ animals.

These studies demonstrate that mice acutely infected with Armstrong strain of LCMV make interferon and that acutely infected T cell-deficient mice make greater amounts of interferon, particularly at the sixth and seventh days after initiation of infection, concomitant with their survival. It has been shown by various depletion and reconstitution studies that T-derived lymphocytes sensitised to LCMV (refs 14-16) as well as antibody to LCMV and complement¹⁷ are associated with tissue injury in acute or chronic LCMV infections¹³. The transfer of sensitised spleen cells¹⁸ is associated with significant decreases in viral

Table 1 Interferon and virus titres after LCMV infection of BALB/c, Nu/Nu and Nu/+ mice

Organ	Day after infection	Interferon and virus titres		
		Nu/Nu	Nu/+	BALB/c
Spleen	4	2,000*	2,580	1,400
	6	4,100	130	100
	7	8,250	271	50
		(5.9×10^6)†	(8.8×10^4)	(1.24×10^4)
Liver	4	250	456	70
	6	1,200	1,100	800
	7	170	70	70
		(5.8×10^4)	(2×10^3)	(2×10^3)
Kidney	4	200	220	24
	6	215	180	160
	7	120	20	15
		(2.4×10^3)	(8.4×10^3)	(10^3)
Brain	4	20u	10	20
	6	35u	75	25
	7	20u	35	15
		(7.3×10^7)	(1.5×10^7)	(5.8×10^7)
Serum	4	1,400	1,650	460
	6	2,150	100	30
	7	4,150	205	60
		0%(0/10)‡	100%(10/10)	100%(10/10)
Mortality	10			

Acute infection was induced at 6 weeks of age with 100 LD₅₀ of LCMV i.c.

*Units of antiviral activity per gram of tissue or ml of serum as determined by titration of a pool obtained from five mice.

†Figures in parentheses represent virus titre in PFU per gram of organ as measured by BHK cell monolayers (results are an average of five individual single organ titrations).

‡Animals surviving/animals challenged. No homozygote nude mouse died of acute infection. With both heterozygotes and parental strains, approximately 50% died by day 7, 80–90% by day 8, and all by day 10.

titres and transfer of sensitised spleen cells¹⁸ or depletion of complement¹⁷ is associated with enhanced survival, although the bulk of evidence indicates that T-derived lymphocytes have a major role in survival and/or immunopathological injury. The observations reported here suggest that high tissue interferon levels in the nudes may perhaps also have a protective role in this virus infection. With recent observations^{19–24} of an immunoregulatory action of interferon, it is possible that interferon may directly modulate some of the altered immunological responses observed in LCMV infected nude compared with controls. Unfortunately, our findings provide no direct evidence for this possibility. In addition, we found that BALB/c and Nu/+ mice dying of acute LCMV infection have high titres of virus in their brain tissues together with low levels of interferon and abundant tissue injury. The latter results contrast with the high titres of both interferon and virus found in other tissues and modify the alternative interpretation that interferon production is just a concomitant of high concentrations of LCMV.

The antiviral substance we have observed in this report and have identified as interferon should be differentiated from the inhibitor of virus replication observed by Veltri and Kirk²⁵ in animals acutely infected with LCMV. Their inhibitor was present in higher titre in the brain than in the spleen and was inactivated by pH 2 exposure. It also demonstrated activity on human cells. Furthermore, the action of their inhibitor did not persist in the treated cells and was eliminated with a media change whereas ours was not.

In summary, our studies have shown that nude mice have higher titres of interferon in their spleens and serum during LCMV infection concomitant with higher virus titres in various tissues and their survival from this infection. The significance of increased interferon production in the survival of these animals remains to be determined. Their survival is undoubtedly favoured by their inability

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Immunological tolerance and tumour allografts in the brain

THE brain has been considered to be an immunologically privileged site with regard to tissue transplantation. In addition, a loss of 'tumour surveillance' due to a weakening of the immune system in the brain has been postulated. The data on the brain as a privileged site are not unequivocal, however. The evidence for and against this thesis has been discussed by Woodruff¹ and Lance². Barker and Billingham³ also speculated that "reports that the brain can prevent implanted homografts from inciting sensitivity because it lacks a lymphatic drainage, thus having no afferent pathway of the immunological reflex, are more equivocal". The need for a critical re-evaluation of the brain as a privileged site has been stressed. We report here a study of the possible action of immunological tolerance in the brain and of the sensitivity of normal rats and tolerant rats to inoculation of allogeneic tumour cells into the brain. We show that transplantation tolerance is involved even in the brain; thus demonstrating the presence of specific immunity following inoculation of tumour cells into the brain. This finding suggests that previously held views of the brain as a privileged site may not be entirely valid and that specific immune processes are accomplished in the central nervous system.

AVN strain rats were used as recipients. The donors were Lewis strain rats, differing from the AVN recipients in H-1 plus non-H-1 antigens. Immunological tolerance was induced in newborn AVN recipients by the intravenous injection of $40\text{--}45 \times 10^6$ bone marrow cells from Lewis donors. Skin grafts from Lewis rats were transplanted 2 months after birth. Tumour cells which were inoculated into the brains were obtained from the culture of a virogenic sarcoma RSL. The RSL sarcoma was induced in a newborn Lewis rat by injection of the Schmidt-Ruppin strain of Rous sarcoma virus⁴. $5 \times 10^3\text{--}5 \times 10^6$ tumour cells

in a volume of 0.05 ml of Eagle's medium were injected with a microsyringe into the occipital lobe in the right hemisphere 1.5–2 cm in depth of 4-month-old tolerant and untreated control AVN recipients and syngeneic Lewis recipients. After tumour cell inoculation, the animals were inspected daily and the effect of tumour growth was evaluated by death of the animals. Some animals were taken for histological examination.

The results show that the action of transplantation tolerance extends even to the brain. While untreated animals were resistant to tumour cell inoculum, except for the largest dose of 5×10^6 cells, all of the tolerant animals, like the syngeneic recipients, died after even the smallest dose of 5×10^3 cells (Table 1). Some of the non-tolerant animals died within 5–11 d of allotransplantation of the largest dose of tumour cells, whereas deaths in tolerant and syngeneic recipients of the smaller doses occurred at a slower rate (Table 2). The resistance of untreated animals

Table 1 Mortality of allogeneic normal and tolerant rats, and of syngeneic rats, following inoculation of tumour cells into the brain

No. of injected tumour cells from Lewis rats	Allogeneic AVN recipients		Syngeneic Lewis recipients
	Untreated	Tolerant	Untreated
5×10^3	0/10*	5/5	9/9
5×10^4	1/10	8/8	NT†
5×10^5	0/10	NT	NT
10^6	0/20	10/10	5/5
5×10^6	14/24	NT	NT

*Numerator, number of dead animals; denominator, total number of animals.

†NT, Not tested.

to the inoculated tumour allografts was immunologically specific, as it was abolished by the induction of allogeneic tolerance. When large doses of tumour cells were inoculated into the brain some non-tolerant recipients died. RSL tumour cells injected into the muscle of the hind feet of adult AVN rats in amounts up to 10^7 cells did not induce distinct tumour growth. In 2-month-old rats the dose of 10^7 cells gave rise to tumours, but these regressed within 16 d and no animals died.

Thus, tumour tissue allografts grew relatively better in the brain than in other sites; this seems to agree with the findings of Murphy and Sturm⁵, who investigated the survival of tumour xenografts. Our observations that tumour allografts in the brain are more fatal than those in limb muscle may be, however, due to the fact that the tumour growing in the brain kills the animal before a sufficiently intense immune reaction develops. That is, the comparison may not be quite fair as the anatomical difference may simulate an immunological privilege.

Histological findings in normal animals showed the presence of lymphocytes in the brain tissue, and these cells could have participated in the immune reaction evoked by injected tumour cells. In the proximity of tumour cells, infiltrates of lymphocytes were observed in the perivascular spaces. The immune reactions in the brain were demonstrated in the experiments of Šterzl and Lodin⁶ in which

Table 2 The mortality range of allogeneic tolerant and syngeneic rats following inoculation of tumour cells into the brain

Recipients	No. of inoculated tumour cells		
	5×10^3	5×10^4	10^6
	Deaths on days		
Allogeneic tolerant	17–21	12–16	6–10
Syngeneic untreated	17–21	NT*	6–9

*NT, not tested.

the administration of antigen to the brain led to marked antibody formation by the immigrating cells localised in the brain. Thus, it seems that the vascularisation of the brain is sufficient to mediate the afferent and efferent arcs of the immunological reaction. Consistent with this notion are the speculations of Tilney and Gowans⁷ who posed the question "if kidneys can sensitise their hosts by way of the blood, why cannot fully vascularised grafts in immunologically privileged sites do the same?" The vascularisation itself has an important role in the survival of the tissue implants in the brain⁸. But it is difficult to explain the long-term survival of allogeneic normal or tumour tissues, as observed by some investigators (see refs 1 and 2 for reviews). The avascularity of the implants might be the cause. Although the concept of the brain as an immunologically privileged site is still, to some extent, in dispute, the data reported here clearly show that after tumour inoculation the basic immunological reactions are carried out in the brain.

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Macrophages isolated from regressing Moloney sarcomas are more cytotoxic than those recovered from progressing sarcomas

THERE is increasing evidence that many tumours contain appreciable numbers of macrophages^{1–3}, and that these inflammatory cells can be cytotoxic for various neoplastic cells *in vitro*^{4,5}. Thus at first it seems paradoxical that many tumours are progressive and lethal in spite of a high content of macrophages. But to kill a malignant cell, these inflammatory cells must be brought to an ill-defined state of 'activation'^{6,7}. It is therefore conceivable that the macrophages in progressing tumours are less activated, and thus less cytotoxic, than those in spontaneously regressing neoplasms. To test this hypothesis we isolated macrophages from either regressing or progressing Moloney sarcomas and tested them *in vitro* for their capacity to release chromium-51 (⁵¹Cr) from prelabelled target cells. The former were usually significantly more cytotoxic than the latter.

At the times of collection (7–13 d after inoculation) regressing tumours (regressors) were 0.3–0.5 cm in diameter, firm and somewhat difficult to distinguish from adjacent normal skeletal muscle. By contrast, progressing tumours (progressors) were 1–2 cm in diameter, soft and friable and contained no grossly identifiable muscle. There were small foci of recent haemorrhage in the centres of the larger

tumours. Progressors, left to run their course, kill the host after metastasising to the lung^{8,9}, while regressors are rejected completely, usually by the fourth week after inoculation^{3,8}. Regression is attended by an extensive mononuclear inflammatory response^{8,10}, shown in tumour disaggregation studies to consist principally of T lymphocytes and macrophages³. Both these inflammatory cell types are also found associated with progressors, but unlike regressing sarcomas, inflammatory cells do not penetrate beyond the peripheries of progressing neoplasms.

Tumours were disaggregated as before¹¹, and macrophage monolayers were established from the resultant cell suspensions¹². Macrophages in monolayers were counted in Giemsa-stained monolayers after exposure to zymosan particles. As measured by release of ⁵¹Cr (details in legend for Table 1), at a density of 1,500 mm⁻², macrophages recovered from regressing Moloney sarcomas killed both the P815 mastocytoma and SV40-transformed 3T3 target cells (Table 1), albeit with different efficiencies (probably reflecting differences in target cell susceptibility). By contrast, neither target cell was affected by macrophages collected from progressors. Similar results at this macrophage density have been obtained in three experiments.

The density of macrophages in monolayers is of critical importance in demonstrating their cytotoxicity. Several

groups have shown, for example, that maximal killing by these cells is not obtained unless monolayers are confluent, or nearly so. Doe and Henson (unpublished results) have investigated this aspect of macrophage-mediated cytotoxicity in detail, using the basic assay system described here. When thioglycollate broth-induced peritoneal macrophages were activated by bacterial lipopolysaccharide, Doe and Hensen obtained no measurable cytotoxicity at monolayer densities of <3,000 mm⁻², while maximal effects (that is, >80% specific ⁵¹Cr release) were obtained at macrophage densities of 5,000 mm⁻² or more. In view of these findings it is clear that the experiments we have described, where monolayers were 50–60% confluent, did not distinguish whether progressor macrophages were absolutely unable to cause ⁵¹Cr release or, rather, that they were simply diminished in their cytotoxic capabilities compared with regressor macrophages. We therefore addressed this point by establishing, for regressors and progressors, a series of monolayers of increasing macrophage density (details in legend for Fig. 1). Similar results were obtained in approximately 80% of all such experiments (postinoculation days 7–13),

Table 1 Cytotoxicity of macrophages recovered from regressors or progressors

Target cell	Source of macrophages	c.p.m. in supernates of triplicate cultures (mean ± s.d.)	% Corrected ⁵¹ Cr release
P815 mastocytoma	None	2,152 ± 11	—
	Progressor	2,163 ± 65	0.2
	Regressor	4,127 ± 35	32.0*
SV40-transformed 3T3 cell	None	1,808 ± 14	—
	Progressor	1,798 ± 56	-0.2
	Regressor	2,247 ± 92	10.0*

Regressing or progressing Moloney sarcomas were induced in 6–8-week-old, male, BALB/c mice by intramuscular injection of either 5 × 10³ or 10⁶ cultured MSC cells, respectively^{3,8}. Tumours collected 13 d later were disaggregated using a stirred mixture of trypsin, collagenase (each 0.1 mg ml⁻¹) and DNase (0.025 mg ml⁻¹) (ref. 11). Macrophages in resultant cell suspensions were plated in 16-mm diameter, flat bottomed wells of plastic tissue culture trays (Linbro). Monolayers (50–60% confluent) containing 3 × 10⁵ cells per well (1,500 per mm²) (determined by cell counting using an inverted microscope equipped with a gridded ocular of known area) were obtained by brief exposure of each well twice to 2.5 × 10⁶ cells from the crude suspension in 0.5 ml HEPES-buffered (15 mM) minimum essential tissue culture medium (HMEM) containing 10% foetal bovine serum (FBS). Five minutes after each seeding plates were agitated and all non-adherent cells were washed away after a total of 10 min. Ninety per cent of the adherent cells from both regressors and progressors were macrophages, as determined by microscopic examination of representative, Giemsa-stained monolayers treated and untreated with zymosan particles. The principal contaminants were tumour cells and polymorphonuclear leukocytes. Target cells antigenically unrelated to MSC cells, and therefore insensitive to killing by the rare T lymphocyte which may have contaminated effector cell populations, were labelled for 1 h in HMEM+10% FBS containing sodium ⁵¹chromate (250 μCi ml⁻¹, Amersham/Searle, Arlington Heights, Illinois; specific activity 200 mCi mg⁻¹). After washing, targets were allowed to stand at 37 °C for 2 h (with agitation at 30-min intervals), counted, resuspended in HMEM+30% FBS and added to wells (10⁵ each; macrophage-to-target cell ratio 3:1). After incubation at 37 °C for 17 h, half of each culture supernate, collected by centrifugation, was assayed for radioactivity in an automatic gamma scintillation spectrometer. Percentage corrected ⁵¹Cr release was computed using the formula ((experimental ⁵¹Cr release - spontaneous ⁵¹Cr release) ÷ (freeze-thaw ⁵¹Cr release - spontaneous ⁵¹Cr release)) × 100. Negative values indicate that release in experimental cultures was less than spontaneous release. Rates of spontaneous ⁵¹Cr loss by P815 and SV40-transformed 3T3 cells were 1.5% and 1.7% per h, respectively.

*As determined by Student's *t* test, the differences in cytotoxicity between regressor and progressor macrophages are significant at the <0.01 (SV40 3T3) and <0.001 (P815) levels.

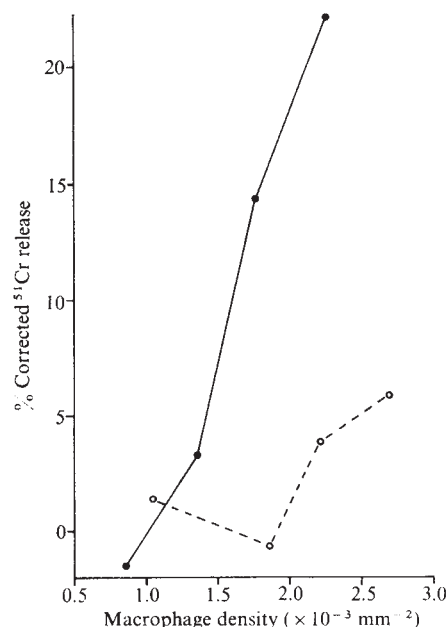


Fig. 1 Cell suspensions were prepared from either regressing (●) or progressing (○) Moloney sarcomas (postinoculation day 8) and plated as described under Table 1 and in ref. 12. Numbers of macrophages were increased by repeated exposures of Linbro wells (200 mm²) to the crude cell suspension. The lowest density was obtained by a single exposure of 5 min, the second by one exposure of 10-min, the third by two 10-min periods, and the highest by three 10 min incubations. The wells were washed after each exposure. Specific ⁵¹Cr release was assayed as described under Table 1.

suggesting that progressor macrophages can kill, but that their relative cytotoxicity is much less than that of macrophages recovered from regressors. There was some variation, however, for occasionally (3 per 14 experiments) progressor macrophages gave a level of killing nearly as high as or, in one case, higher than that obtained using regressor macrophages. In contrast to the small amount of killing usually obtained with progressor macrophages, such results were obtained sporadically and were not reproducible. The cause of such variation may be technical or stem from some undefined variable associated with the tumour system.

Thus it seems that macrophages recovered from regressing Moloney sarcomas generally are more cytotoxic than those obtained from progressing neoplasms of the same type. There is precedent for this finding in the work of

Fidler⁶ and G. Miller and J. Feldman (personal communication). In both cases peritoneal macrophages induced by injection of thioglycollate broth were cytotoxic if the animal from which they came had been pre-immunised against the tumour target cell used, but were unable to kill or were greatly diminished in this capacity, if collected from an animal which carried a progressing tumour induced by the same target cell. Fidler's work further suggested that the observed inability to kill was not an innate deficiency of the macrophage, but was due to lack of macrophage activation by soluble lymphocyte mediators. One possible explanation for the diminished cytotoxicity exhibited by progressor macrophages therefore is inadequate activation of these cells by intratumoral lymphocytes. Indirect support for this hypothesis is found in the recent demonstration that, in at least one regard (direct mediation of cytotoxicity), T lymphocytes recovered from large progressing Moloney sarcomas were functionally inactive¹³. Direct testing of the macrophage activating potential of progressor T lymphocytes is in progress.

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Aspartic acid racemisation in the human lens during ageing and in cataract formation

D-ASPARTIC acid has been shown to accumulate with age in human tooth enamel¹ and dentine² at a rate of about 0.1% yr⁻¹. We have predicted that racemisation should take place in any metabolically stable protein in long-lived mammals and that, as a consequence of racemisation, these proteins will have altered conformations which would probably produce changes in their biological activities or chemical properties³. We have extended these studies to soft tissue proteins, in particular those in the human lens. The proteins in the central portion of the lens are among the most stable in the human body⁴. Numerous changes in the properties of the lens proteins occur with age and cataract formation—denaturation⁵, increasing pigmentation⁶, cross linking^{5,6}, insolubility⁶⁻⁹, and fluorescence¹⁰. Pirie¹¹ has suggested that physicochemical processes may be responsible for these changes. We report here the results of D/L enantiomeric analyses of normal

human lenses and cataracts: aspartic acid racemisation was seen during ageing and cataract formation.

Human lenses were obtained from Eye Bank collections or after surgical extraction at the Duke University Medical Center. Cataracts were graded according to the colour classification of Pirie⁶: group I, pale yellow lens; group II, pale cortex with visible nucleus; group III, pale cortex with hazel brown nucleus; group IV, pale cortex with deep brown nucleus. The central 15% by weight of each lens was removed by the method of Spector *et al.*¹⁰; these samples will be referred to as the central nuclei. A freshly extracted cataract lens from a 76-yr-old individual was sectioned as above. Then two additional samples were taken from the remainder of the lens, one representing the inner cortex and a second from the peripheral cortex. All lens samples were stored frozen.

The samples were processed using procedures described elsewhere^{12,13}. Measurements of D/L aspartic acid ratios were performed on a Beckman-Spinco Model 118 automatic amino acid analyser, using the diastereomeric dipeptide technique¹⁴. The D/L enantiomeric ratios for glutamic acid, proline, alanine, phenylalanine, and leucine were determined by gas chromatography. The *N*-trifluoroacetyl-L-prolyl peptide methyl esters were synthesised, and separation was performed by gas chromatography (Hewlett-Packard Model 5711A with flame ionisation detector; 20-foot glass column packed with 8% SP 2250 coated on Chromosorb W-AW-DMCS solid support¹⁵).

The D/L aspartic acid analyses for the central nucleus samples of 17 normal human lenses are plotted in Fig. 1. The line in Fig. 1 represents a least squares fit to the normal lens data, and the equation for this line is

$$\ln\left(\frac{1+D/L}{1-D/L}\right) = (2.50 (\pm 0.29) \times 10^{-3} \text{yr}^{-1})(t + 0.112) \quad (1)$$

where *t* is the age of the individual. The data are presented in the form of the reversible first order rate equation¹⁶, rather than the irreversible equation used for the enamel and dentine results^{1,2}, because D-aspartic acid is present

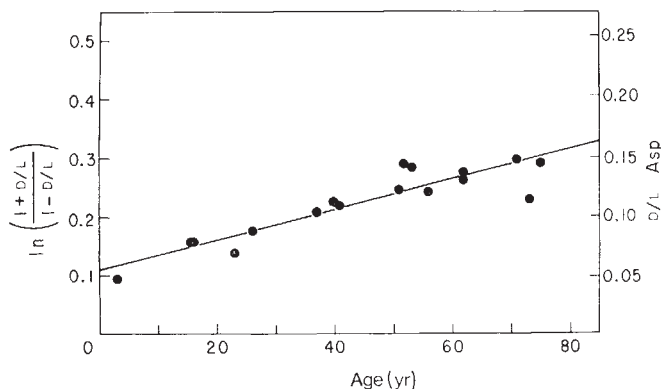


Fig. 1 Analysis of D/L-aspartic acid contents of central nucleus samples of 17 normal human lenses.

in sufficient concentrations in the lenses to make the use of the irreversible first order rate equation invalid. The slope in equation (1) corresponds to $2k_{asp}$. Thus, in the lens nucleus, $k_{asp} = 1.25 \times 10^{-3} \text{yr}^{-1}$. The correlation coefficient (*r*) for the lens results is 0.912.

The y-intercept, 0.112, is equivalent to a D/L ratio of 0.056. This ratio is higher than the enamel¹ (0.033) and dentine² (0.017) *t* = 0 values. A possible explanation for this difference is that the primary structures of proteins

may influence the amount of acid-catalysed racemisation which occurs during the hydrolysis step¹⁴.

Since the proteins in the central nucleus are thought to be 'older' than the proteins in the peripheral regions of the lens, the D/L-aspartic acid ratios should be higher in the nucleus than in the cortex in samples from the same lens. The D/L ratios obtained for the nucleus, inner cortex, and peripheral cortex of the 76-yr-old cataract are 0.171, 0.167, and 0.047, respectively. Only the peripheral cortex shows evidence of recent protein synthesis. The peripheral cortex result is identical to the D/L ratio from the 3-yr-old lens nucleus, that is, 0.047, and may provide a closer approximation to the $t = 0$ value for human lens proteins than the extrapolated value of 0.056.

Our studies of racemisation rates in teeth^{1,2} led us to propose that D/L-aspartic acid analyses can be used as a biochronological tool to estimate the ages of long-lived mammals. Racemisation in the lens nucleus can be used in a similar manner. There are several advantages to working with lenses rather than teeth for mammalian age determinations. (1) Temperature is probably higher and more constant. (2) Teeth of certain mammals, such as cetaceans, have a rather complex pattern of development in which dentine growth layers are laid down well into adulthood¹⁷. (3) Normal tooth attrition makes sampling from old animals difficult. (4) The faster k_{asp} in lenses permits young ages to be calculated more accurately.

The extent of aspartic acid racemisation in the central nuclei of 13 group I and II cataracts was determined, and a least squares fit of these data yielded $k_{asp} = 1.29 \times 10^{-3} \text{ yr}^{-1}$ ($r = 0.875$), which is indistinguishable from the rate in normal lenses. This is particularly interesting since the cataracts included subcapsular, nuclear, and cortical types associated with different aetiologies or disease states (trauma, diabetes, hypertension, glaucoma, Parkinson's disease and so on). Thus, despite the development of these types of cataracts, the racemisation rate is identical to that observed in the lens nucleus during the normal ageing process.

The brunnescent group IV cataracts listed in Table 1, on the other hand, have a much greater degree of aspartic acid racemisation than we have observed in any stable human proteins. These cataracts have D/L-Asp ratios

No racemisation of these amino acids was observed in any of the lenses. (The expected ratios¹⁸ are at the limits of detection by this technique, however). Thus, only aspartyl residues are racemising to a measurable extent in the nuclear proteins of these lenses, which is the expected result¹⁸. Also, our results suggest that only aspartic acid exhibits an accelerated rate of racemisation in the brown cataract lenses.

If aspartic acid racemisation has a role in biological processes, including ageing, the reaction must occur in a variety of tissues. D-aspartyl residues have been shown to accumulate in normal lens nuclear proteins at a rate of $1.25 \times 10^{-3} \text{ yr}^{-1}$ or 0.14% per year, and therefore aspartic acid racemisation should take place with age in any stable soft tissue protein in long-lived mammals.

The rate constant for the lens nucleus is about 1.5 times the rate observed in tooth enamel and dentine. Although these rate constants fall within two s.d. of each other, several variables could be contributing to the difference in rates. The most obvious explanation is that the temperature of the lens is probably more constant and slightly higher than that of teeth, leading to a more rapid racemisation rate. Alternatively, the different protein environments in calcified and non-calcified tissue could explain the differences. The accelerated aspartic acid racemisation rate associated with brunnescent cataracts cannot be explained by the above factors. Among the possible mechanisms which could give rise to an accelerated rate during cataract maturation are change in the pK_a of the β -carboxyl group due to alteration in the microenvironment within the protein¹⁹ and chelation of the β -carboxyl group by trace metals²⁰.

We have suggested that D-aspartyl residues should alter the conformation of a protein and that these effects will become more pronounced as the D-enantiomer accumulates with age³. A number of studies indicate that structural changes occur in lens proteins with age and in cataracts. The proportion of water-insoluble protein increases with age^{8,9}, and the increase is even more marked in cataracts^{8,9}. The water-insoluble residue can be refractionated with concentrated urea. The urea-insoluble portion is greater in cataracts⁶ and occurs primarily in the lens nucleus⁷. The proteins in cataractous lenses have an increased number of exposed thiol groups and a greater susceptibility to tryptic digestion, which suggests that conformational changes are taking place during cataractogenesis⁵. Cataracts show evidence of more disulphide^{20,21} and non-disulphide⁷ covalent cross links than do normal lenses, and the cross links increase during cataract maturation. It is possible that aspartic acid racemisation may actually be induced by structural changes in the lens proteins initiated by other factor(s). But, the above observations are also consistent with the predicted consequences of the racemisation of aspartyl residues.

We do not know whether the accelerated rate of racemisation of aspartic acid contributes to the development of brunnescent cataracts or whether during cataract formation the aspartyl residues become more susceptible to racemisation. Since the percentage of urea-insoluble protein increases more than fourfold between yellow and group IV cataracts²², protein alteration seems to coincide with the increase in racemisation rate.

Regardless of the actual mechanism responsible for the accelerated rate of aspartic acid racemisation in cataract maturation, this chemical alteration provides important information on processes taking place in the human lens during the development of a pathological condition. Elucidation of this mechanism may provide new insights into the process of cataract maturation.

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Table 1 Extent of aspartic acid racemisation in brunnescent cataracts

Age (yr)	D/L-aspartic acid	
	Expected*	Observed
57	0.127	0.203
76	0.150	0.296
77	0.151	0.268
80	0.155	0.283
89	0.166	0.262

*The expected ratios were calculated from equation (1) using the age of each individual.

58–97% higher than either the normal lenses or group I and II cataracts of similar ages. One yellow cataract of unknown age also had a very high D/L ratio of 0.265, which is equivalent to the brunnescent lens values, while a group III cataract (76 yr. D/L Asp = 0.171) falls within the range of the yellow cataracts. Therefore, enhanced aspartic acid racemisation is strongly but not invariably associated with the development of brown pigment during cataract maturation.

Gas chromatographic analyses were performed on two normal (3- and 75-yr-old), seven yellow, and three group IV lenses to determine the D/L enantiomeric ratios for alanine, glutamic acid, proline, phenylalanine and leucine.

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A role of selenium against methylmercury toxicity

SINCE Parizek *et al.*¹ reported that administration of selenite clearly decreased toxicity of mercuric chloride, there have been many studies on the mechanisms responsible for the interrelationship between selenium and inorganic or organic mercury. Almost all have shown that inorganic selenium, especially selenite, prevents growth inhibition as well as mortality and neurotoxicity due to methylmercury given simultaneously in the diet of Japanese quail²⁻⁴ and rats⁵⁻⁸. Protective effects of selenium against methylmercury toxicity do not involve mercury absorption through intestines⁹ or excretion in the urine and faeces⁶. Selenite does not increase the rate of breakdown of methylmercury⁷, and thionein does not have a significant role in the detoxification of methylmercury³, but presumably, the form of methylmercury is modified in some way by selenium. We present here evidence that selenite can release methylmercury from its linkage with proteins and thereby influence its tissue distribution.

The association constants of methylmercury for sulphur ligands of the sulphhydryl group of proteins are extremely large¹⁰. But methylmercury in animal tissues can be separated from Hg-S linkage by hydrochloric acid (1 M or 2 M) and extracted with benzene, then analysed, using a gas chromatograph with an electron capture detector¹¹. Methylmercury extracted with benzene in acid conditions is defined as total methylmercury, methylmercury extracted in neutral conditions as free methylmercury and the difference between them as bound methylmercury.

Selenium compounds in phosphate buffer (pH 7.4) were added to methylmercury and defibrinated human blood and methylmercury was extracted with benzene after 2 h of incubation. Selenite showed a surprisingly high ability to release methylmercury from blood proteins and the effect of selenate is one-tenth that of selenite (Fig. 1). Na₂SO₄, Na₂SO₃, Na₂H₂TeO₆ and Na₂TeO₃ were not effective in inducing release of methylmercury.

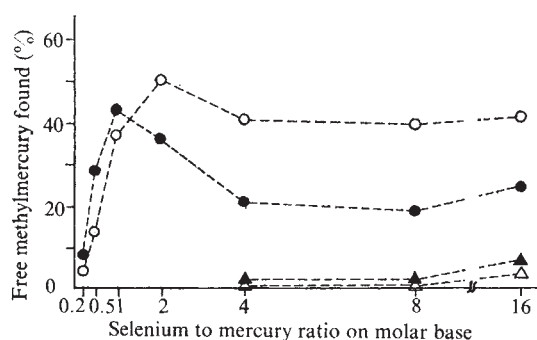


Fig. 1 Release of methylmercury from human blood by Na₂SeO₃ or Na₂SeO₄. A mixture of 0.5 ml of defibrinated blood, 5 nmol methylmercury chloride and selenite or selenate in 5 ml of phosphate buffer (pH 7.4) was incubated for 2 h and free methylmercury was extracted in 2 ml of benzene. The benzene layer was analysed by a gas chromatograph with electron capture detector of ⁶³Ni in the following conditions: 3 mm × 1 m glass column of Thermo 1000 on Chromosorb W at 80 ml min⁻¹ of N₂ flow rate and 180 °C. ○, Na₂SeO₃ at 37 °C incubation temperature; ●, at 20 °C; △, Na₂SeO₄ at 37 °C and ▲, at 20 °C.

Addition of selenite and blood was effective in releasing methylmercury bound to bovine serum albumin, human serum, cysteine and glutathione. Addition of serum had no effect, but red blood cells effectively induced methylmercury release and did not lose effectiveness even if haemolysed by water or washed by benzene; however, they lost this ability when coagulated by heating or denatured (for example by alcohol).

Red blood cells and liver, kidney and brain homogenates from rats (Fig. 2) and mice, and muscle homogenates of raw tuna were also able to release methylmercury in conjunction with selenite. The amounts of free methylmercury released by active agents were proportionate to incubation time, amounts of biological sample, and incubation temperature. The larger the sample size and the higher the temperature, the sooner the maximum point tended to be reached. This occurred in both homogeneous and heterogeneous systems, and also in all cell fractions of mitochondria, microsome and supernatant fractions at 100,000g. The optimal pH was about 6.5 in conditions detailed in Fig. 1, with different phosphate buffers. The ratio of Se to Hg in the benzene layer was not always constant. The value of methylmercury at molar base was

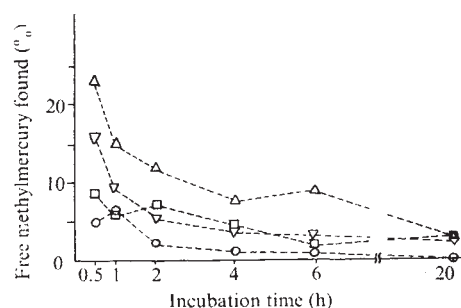


Fig. 2 Release of methylmercury from albumin by tissue homogenates and Na₂SeO₃. Methylmercury chloride (50 nmol) and 2 ml of 1% egg albumin were mixed and 50 nmol of sodium selenite and 1 ml of rat tissue or blood homogenate (each homogenate composed of one third tissue or blood and two thirds phosphate buffer) were added. Enough phosphate buffer was added to bring the total volume to 50 ml, and the mixture was incubated at 37 °C. Free methylmercury was separated from 5-ml aliquots with 2 ml benzene. □, Liver; ▽, kidney; △, brain; and ○, blood.

Table 1 The effects of sodium selenite on the *in vivo* release and tissue distribution of methylmercury

Group	No. of mice	Blood		Liver		Methylmercury content Kidney		Brain	
		TM (p.p.m.)	FM(%)	TM (p.p.m.)	FM(%)	TM (p.p.m.)	FM(%)	TM (p.p.m.)	FM(%)
I, Hg (control)	17	1.13±0.27	<0.1	4.04±1.49	<0.1	10.5±1.94	<0.1	1.53±0.40	<0.1
II, Hg+Se (i.p.)	17	1.25±0.28	0.14~1.5 (0.43)	3.66±0.77	0.22~2.3 (0.50)	12.4±2.31*	0.37~0.93 (0.59)	1.24±0.25*	<0.1
III, Hg+Se (i.v.)	16	0.90±0.36	0.30~45 (9.1)	3.81±1.10	0.20~7.8 (1.6)	4.45±1.34**	1.1~30 (5.1)	1.80±0.47	<0.1

TM, total methylmercury, and FM, free methylmercury. Values in FM(%) columns are the extreme ranges; figures in parentheses show the median. Group I received 100 µg of methylmercury chloride s.c. and, were killed after 1 week. Group II received 69 µg of sodium selenite i.p., 15 h before killing, which occurred 1 week after methylmercury. Group III received 69 µg of selenite i.v., 30 min before killing, which occurred 1 week after methylmercury.

**P* = 0.05.

***P* = 0.01.

two or more times higher than that of Se estimated by flameless atomic absorption. Moreover, methylmercury from the benzene phase could be removed by cysteine solution, but Se remained largely (about 80%) in the benzene layer. This suggests that selenium may not have been associated with methylmercury. Selenite had a similar effect on methylmercury in the organs of mice treated with methylmercury *in vivo*. There was no evidence to indicate that methylmercury added was changed to dimethylmercury or to inorganic mercury.

In the *in vivo* experiments, dd-strain female mice weighing 25 g were given 100 µg methylmercury subcutaneously. About 1 week later sodium selenite, 69 µg was injected intravenously (i.v.) into the tail vein or intraperitoneally. After the selenite injection, blood samples were collected by heart puncture, and livers (detailed in Table 1), kidneys and brains removed. Blood and tissue homogenates were extracted by benzene both in the absence and presence of hydrochloric acid. After i.v. injection of selenite, free methylmercury was found in blood, liver and kidney but none in brain (Table 1). Total methylmercury content in kidney was also clearly decreased by selenite, but there were no changes in other organs.

It seems reasonable to conclude that the shift in the tissue distribution of methylmercury is a natural consequence of the liberation of methylmercury from sulphhydryl bonds.

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Intermolecular association and geometrical shape as two factors controlling chemical reactions

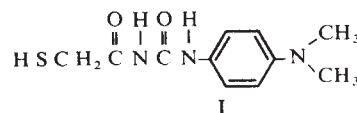
BIOLOGICAL phenomena are highly specific^{1–4} and much effort has been devoted to establishing the relationship between biological activity and molecular shape. We report here the sharp dependence of selectivity in the oxidation of a pair of thiols on their structures or on environment, the mode of each dependence of selectivity being controlled dominantly by their tendency towards intermolecular association.

Two types of model systems were used. One pair of reactants

can associate through intermolecular hydrogen bonding (associating system, system A) and the other pair cannot (non-associating system, system N). The model compounds (I and II) for system A are composed of three regions—the reaction site (SH group) where oxidation takes place, the binding site



an acylurea bond that can participate in intermolecular hydrogen bonding⁵, and the recognition site (–C₆H₄N(CH₃)₂ or R¹) that may further participate in the discrimination. A pair of simple thiols, ethyl thioglycolate (III)

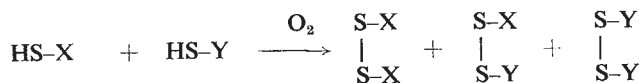


III



IV

and alkanethiol (IV, R² = (CH₂)_kCH(CH₃)₂), were used as the model compounds for system N. A 1:1 mixture of I and II (or of III and IV), on treatment with oxygen, gave one unsymmetrical and two symmetrical disulphides. The selectivity factor (*R*) in this oxidation (an indication of recognition of II by I—or of IV by III), is represented by the ratio (V/VI = *R*) of unsymmetrical disulphide (V) to symmetrical disulphide (VI) obtained from I or III.



I or
III

II or
IV

VI

V

Oxidation of a mixture of methanethiol and ethanethiol is known to yield the three disulphides in a 1:2:1 ratio⁶. This was also the case with oxidation of system N in 80% aqueous acetonitrile at 35 °C: the product ratio (*R*) was 2.1 in two cases (*k* = 1 and 2, Fig. 1). In contrast, quite different results were obtained with system A. When a series of branched alkyl groups ((CH₂)_mCH(CH₃)₂) were used as R¹, selectivity showed a maximum (*R* = 21.2, *V* = 87%) at *m* = 2. Such a remarkable dependence of selectivity on the substituent R¹, however, was not observed for system A where a series of straight-chain alkyl groups ((CH₂)_nCH₃) were used as R¹:

the product ratio (R) changed slightly from 0.3 to 0.5 except for the case $n = 2$. The data in Fig. 1 suggest the importance of intermolecular association in controlling chemical reactions through small changes in the structures of reacting molecules.

Figure 2a illustrates the plots of R at 35 °C against dielectric constants (ϵ), a measure of polarity of solvents. Figure 2a shows that with the associating system having the isopentyl group as R^1 in II (system A_a), selectivity decreased in going from 80% to 90% and 70% aqueous acetonitrile and from 80% acetonitrile to 80% dioxane and 80% N,N -dimethylformamide (DMF) with the maximum at 80% acetonitrile ($\epsilon = 42.6$). The marked dependence of selectivity on ϵ led us to examine whether the thiol I or II associates in benzene or in acetonitrile—using a differential vapour pressure method. The degree of association (f), obtained by dividing the stoichiometric mole fraction of the solute by the effective mole fraction of the solute, was 1.31, 1.80, and 1.77 in benzene at 36 °C for

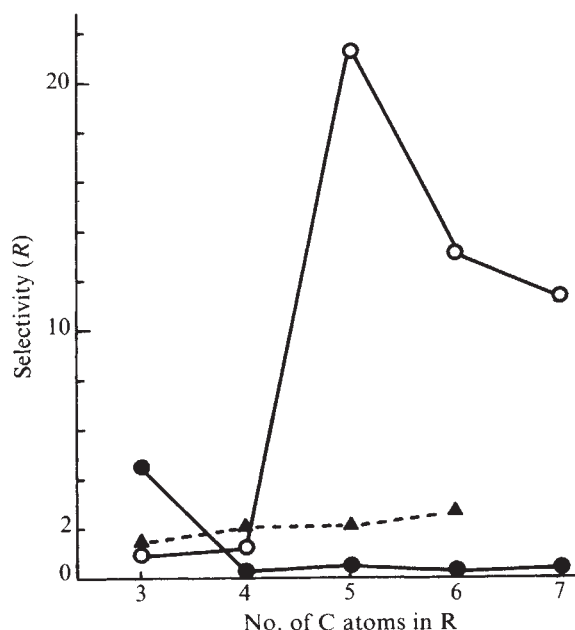


Fig. 1 Dependence of selectivity (R) in oxidation of a pair of the thiols (I and II or III and IV) on the structures of R^1 or of R^2 . The two thiols (0.5 mmol each) were allowed to react with O_2 in 12.5 ml of 80% aqueous acetonitrile (v/v) at 35 °C in the presence of Et_3N (5×10^{-2} mmol) as a catalyst. Satisfactory spectroscopic data were used to support the assignment of new compounds. When the oxidation was completed, yields of V and VI were determined exactly by the use of their absorption at 310 nm after separation by thin-layer chromatography for system A, and determined gravimetrically after separation by preparative thin-layer chromatography for system N. ○, System A (R^1 , branched alkyl groups); ●, system A (R^2 , straight-chain alkyl groups); ▲, system N (R^2 , branched alkyl groups).

I (0.01 M), II_a (0.04 M, $R^1 = \text{iso-C}_5\text{H}_{11}$), and II_b (0.04 M, $R^1 = n\text{-C}_5\text{H}_{11}$), respectively. In contrast, in acetonitrile f was reduced to 1.04, 1.08, and 1.03 at 36 °C for I (0.02 M), II_a (0.04 M), and II_b (0.04 M), respectively. These data clearly indicate that f is reduced with increasing solvent polarity⁷. Accordingly, it is suggested that moderately weak association is essential for effective recognition between molecules, strong association being not essential. When the associating system contained the n -pentyl group as R^1 (system A_b), however, R had a minimum, though selectivity in this case was far less sensitive to ϵ than that with system A_a . In sharp contrast, with the non-associating system with the isopentyl group as R^2 in IV (system N_a) R showed a very slight variation from 2.5 to 1.8. This control experiment demonstrates that in the absence of intermolecular association, the three disulphides are formed in a nearly statistical ratio.

In system A_a a plot of selectivity in 80% acetonitrile against temperature showed a peak of maximum selectivity at 35 °C (Fig. 2b). In system A_b , however, the pattern of temperature dependence did not correspond to that of solvent polarity— R changed progressively over a wide range (0.13–2.3).

Of particular interest is the concentration dependence of selectivity with system A_a in 80% dioxane at 35 °C, represented by a sigmoid-like curve similar to the substrate concentration–reaction rate profiles observed with allosteric enzymes (Fig. 2c). In the case of systems N_a and even A_b , however, R remained almost constant with concentration.

In considering the significance of intermolecular association, it should also be noted that the product ratio (R) approaches about 2, the statistically expected ratio, in all systems in conditions unfavourable for hydrogen bonding, that is, in a polar solvent (for example N -methylformamide, NMF), at a higher temperature (70 °C), or at a lower concentration (1×10^{-3} M). It is clear from the results described here that environment dependence of selectivity is very sharp only in the case of system A_a among the systems examined.

All the results obtained from system A can be said to represent kinetic control on the basis of the following observations. First, the product composition does not change as the reaction proceeds, as is evident from the fact that the yields of V and VI during the period of the oxidation are 6.3% and trace, 15% and about 0.7% ($R = 21$), 23% and 1.1% ($R = 20.9$), 43% and 2.0% ($R = 21.5$), respectively, for system A_a and 2.2% and 4.4% ($R = 0.50$), 4.8% and 9.4% ($R = 0.51$), 14% and 27% ($R = 0.52$), respectively, for system A_b . System A_a required 2.5 h for complete oxidation to give 87% V and 4.1% VI ($R = 21.2$), whereas system A_b required 3.5 h to give 20% V and 39% VI ($R = 0.51$). Second, a thiol–disulphide exchange reaction occurred slowly in 80% acetonitrile at 35 °C in the presence of a catalytic amount of triethylamine under argon. The yields of V and VI at the same reaction time as with the above oxidation were 24% and 22%, respectively, for system A_a ($R = 1.1$, reaction time = 2.5 h) and 3.9% and 40%, respectively, for system A_b ($R = 0.098$, reaction time = 3.5 h), indicating that the product distribution in the exchange reaction differs entirely from that in the oxidation. The disulphide–disulphide exchange reaction did not take place to any appreciable extent in 80% acetonitrile at 35 °C in the presence of a catalytic amount of both I and triethylamine under argon⁸.

System A was heterogeneous in some cases. However, the product ratio could not be ascribed to differential solubilities of the thiols and the product disulphides for the following reasons. First, the experiments on concentration dependence shown in Fig. 2c were carried out in completely homogeneous conditions, but the selectivity showed a sigmoid-like curve in the case of system A_a . Second, in 80% dioxane, 80% DMF, and NMF system A_a was also completely homogeneous at 35 °C during the oxidation, but R varied markedly from 1.9 (Fig. 2a). Third, in 80% acetonitrile a pair of the thiols I and II_a were completely soluble in the 25–70 °C range, the thiols I and II_b being also soluble at 35 and 70 °C (Fig. 2b). Further, the unsymmetrical and symmetrical disulphides derived from I and II were very stable in the reaction conditions, the unsymmetrical disulphides being more soluble in ordinary solvents than the symmetrical disulphides. These findings show conclusively that the selectivity is neither controlled by solubility differences between the thiols and disulphides and between disulphides V and VI nor by whether the reaction is heterogeneous or homogeneous⁹.

Although the detailed nature of the specific interactions between I and II has not yet been made clear, the following observation suggests the presence of such interactions in system A_a . Plots of R against the mole ratio of I and II_a showed a maximum rise of R in a 1:1 mixture of I and II_a, indicating that I and II_a seem to form 1:1 weak complexes with each other, but not to complex with themselves. This type of complex is apparently stabilised by such interactions as those between the

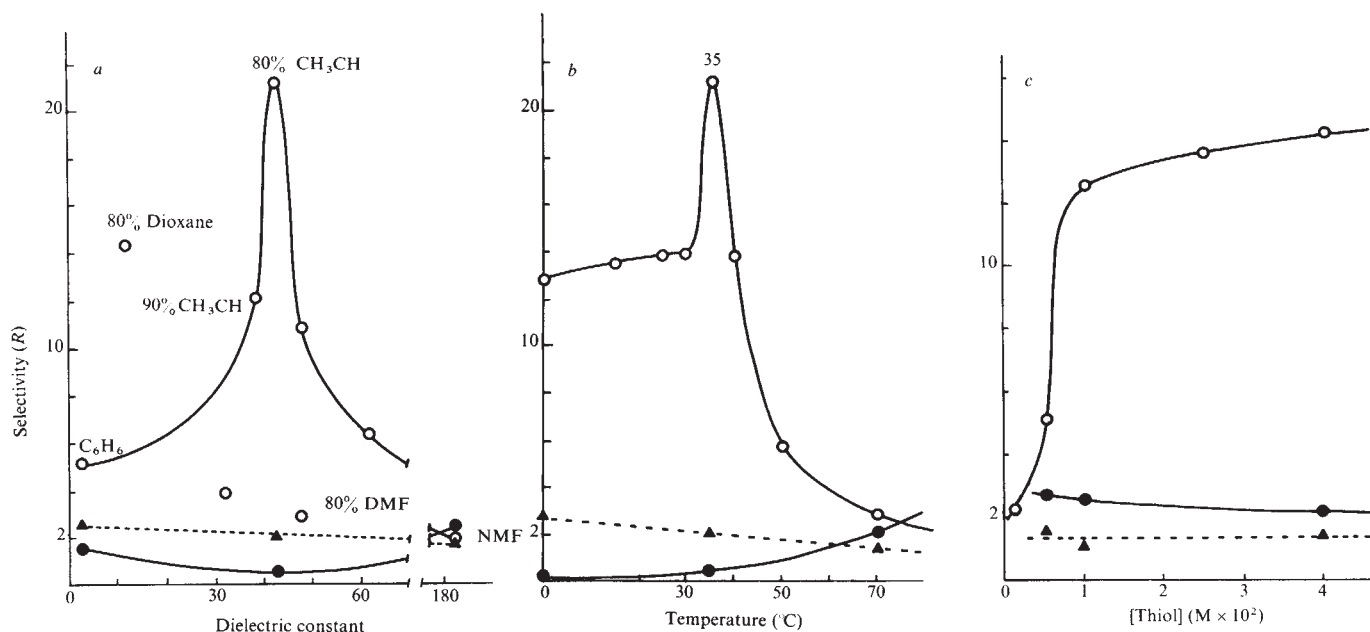


Fig. 2 Dependence of selectivity (R) in oxidation of a pair of the thiols (I and II or III and IV ($R^2 = \text{isoC}_2\text{H}_5$)). *a*, Dielectric constants of solvents (12.5 ml) at 35 °C; *b*, on reaction temperature in 12.5 ml of 80% acetonitrile, and *c*, on concentration of each thiol in 80% aqueous dioxane at 35 °C. The oxidations with O_2 were performed by using the same molar amounts of the thiols and Et_3N as described for Fig. 1. The series of solvents used to study solvent polarity dependence was (in order of increasing dielectric constant): benzene, 80% aqueous dioxane, methanol, 90% and 80% aqueous acetonitrile, 80% aqueous DMF, 70% and 50% aqueous acetonitrile, and NMF. $\circ$, System A_a ; $\bullet$, system A_b ; $\triangle$, system A_c .

aromatic ring in I and the branched alkyl group in II, in addition to intermolecular hydrogen bonding¹⁰ between the two respective acylurea bonds in I and II. We conclude from our studies that both intermolecular association and geometrical shape are required for molecular recognition in chemical reactions.

Our results suggest that the use of appropriate model compounds which can associate and are of relatively low molecular weight should increase understanding of the problem of molecular recognition.

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Effects of cyclic AMP pulses on adenylate cyclase and the phosphodiesterase inhibitor of *D. discoideum*

STARVATION triggers the differentiation of *Dictyostelium discoideum* amoebae to aggregation competence. The differentiated cells orientate and migrate towards attracting centres, presumably consisting of amoebae which autonomously and rhythmically emit cyclic AMP as a chemotactic signal. Signals, emitted with a periodicity of 5–8 min (refs 1–3, 5), are presumably generated by rhythmic changes in adenylate cyclase activity⁶, levels of which dramatically increase during the first few hours before the expression of aggregation competence^{7,8}. Cellular responses to external cyclic AMP are probably mediated by plasma membrane receptors⁹. When stimulated by a pulse of cyclic AMP, amoebae amplify¹⁰ and relay^{1,5,10,11} the chemotactic signal, which results in a coordinated aggregation. The decay of the chemotactic signal is determined by the activity of the extracellular cyclic AMP phosphodiesterase^{12,13} whose activity is modulated by an inhibitor excreted by cells during the early hours of starvation¹⁴. Cyclic AMP pulses have recently also been shown to regulate the differentiation of cells to aggregation competence¹⁵. To determine their mechanism of action, we examined the influence of applied cyclic AMP pulses on the two earliest biochemical events implicated in the control of cyclic AMP levels, the rise in adenylate cyclase activity and the appearance of the extracellular phosphodiesterase inhibitor. The results show that adenylate cyclase may exist in active or inactive forms. Applied cyclic AMP pulses seem to induce cell differentiation by activating the enzyme. They also are shown to repress the production of the phosphodiesterase inhibitor.

The changes in adenylate cyclase and the extracellular phosphodiesterase inhibitor which occurred when amoebae were starved in the presence or absence of cyclic AMP pulses are shown in Fig. 1. These changes are compared with the development of aggregation competence and the

increases in cyclic AMP binding sites^{9,16} and phosphodiesterase^{16,17} which accompany it. In this experiment unpulsed amoebae were aggregation competent after 6–7 h of starvation, when they showed high levels of intracellular phosphodiesterase activity and cyclic AMP binding sites (Fig. 1a). When cells were pulsed with cyclic AMP, high levels of these two parameters were observed after 4.5–5 h of starvation, when they expressed aggregation competence. Assays of adenylate cyclase showed that the time course of enzyme induction was unaltered by cyclic AMP pulses: with pulsed and unpulsed cultures, activity first rose after 2 h of starvation and was maximal after approximately 5 h.

Determinations of the levels of the extracellular phosphodiesterase inhibitor excreted by pulsed cells showed that they produced approximately 4% of the inhibitor produced by untreated cells (Fig. 1b). Incubation of amoebae in buffer containing 10^{-3} M cyclic AMP, delayed the appearance of the inhibitor by 1–2 h. Inhibitor was produced again when the added cyclic AMP had been hydrolysed by the extracellular phosphodiesterase (data not shown). The efficiency of 10^{-7} M cyclic AMP, when administered as pulses, in repressing inhibitor production may suggest that their effects are mediated by the increased cellular cyclic AMP levels obtained during signal amplification.

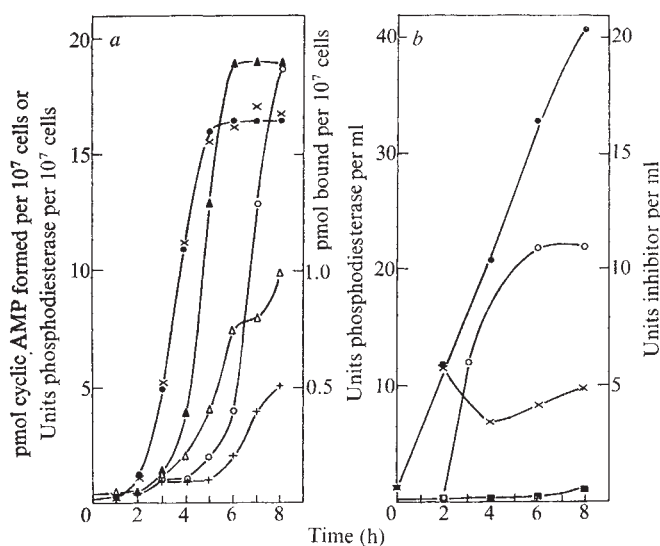


Fig. 1 Changes in adenylate cyclase, cyclic AMP binding sites, phosphodiesterase, and the phosphodiesterase inhibitor in cyclic AMP-pulsed and -unpulsed cells. Ax2 amoebae, harvested during exponential growth in HL-5 media²¹, were washed twice with 17 mM phosphate buffer pH 6.2 and starved in that buffer as agitated suspensions²² at a density of 10^7 cells ml⁻¹. Treatment of amoebae with 10^{-7} M cyclic AMP pulses was begun immediately after transfer of cells into buffer (t_0). The technique has been described in detail elsewhere¹⁵. Control cells were starved in the absence of applied pulses. At the indicated times, aliquots were taken and assayed for adenylate cyclase⁷, cellular and extracellular phosphodiesterase activities¹⁷ and the levels of cyclic AMP binding sites²³ and extracellular phosphodiesterase inhibitor¹⁴. Preparation of samples for inhibitor assays involves heating for 10 min at 80 °C (ref. 14). Although this treatment has been shown to result in a quantitative recovery of the inhibitor (independent of its interaction with the extracellular phosphodiesterase)¹⁴, this was verified further for our experimental conditions where phosphodiesterase was present in large excess of inhibitor. Units of phosphodiesterase activity are defined as nmol cyclic AMP hydrolysed per min at 30 °C. The differentiation of cells to aggregation competence was followed by plating aliquots of cells on to Falcon tissue culture dishes at hourly intervals and observing cell morphology and formation of EDTA resistant cell contacts²². a, Adenylate cyclase of control cells (○) and of pulsed cells (●); cellular phosphodiesterase of control cells (○) and of pulsed cells (▲); cyclic AMP binding sites of control cells (×) and of pulsed cells (△). b, Extracellular phosphodiesterase of control cells (○) and of pulsed cells (●); phosphodiesterase inhibitor of control cells (○) and of pulsed cells (■).

Extracellular phosphodiesterase levels increased 4–5-fold during the time cells were stimulated by cyclic AMP pulses (Fig. 1b). This increase results from both a rise in enzyme production¹⁷ and the decrease in the levels of the enzyme inhibitor. The dual control of cyclic AMP pulses on phosphodiesterase and its inhibitor assures the levels of extracellular phosphodiesterase required for cell differentiation (M.D. *et al.*, in preparation). Since amoebae feed on bacteria and therefore do not all begin to starve at the same time, the presence of the inhibitor during the early hours of starvation could assure that no aggregation occurs until the majority of the cell population produces the chemotactic signal. At that time, inhibitor production would be arrested and the phosphodiesterase induced.

The results described in Fig. 1 suggest that the rise in adenylate cyclase is not directly correlated with the expression of aggregation competence. This is further supported by the observation that unpulsed cell populations, which became aggregation competent after different time periods (5, 8 or 12 h), all increased their adenylate cyclase activities during the first few hours of starvation. In all these cases, applied cyclic AMP pulses did not alter the time course of the changes in adenylate cyclase but shortened the interval between the increase in this activity and the expression of aggregation competence and its related biochemical parameters. Cells may maximally increase their levels of adenylate cyclase during the first few hours of starvation but not display aggregation competence until many hours later because the enzymic activity assayed in cell lysates

Table 1 Cyclic AMP levels of late and early differentiating amoebae

Time of starvation (h)	Cyclic AMP (pmol per 2×10^7 cells)	
	Experiment 1	Experiment 2
2	0.17	3.5
4	0	4.0
5	.01	5.5
6	0	4.2

To initiate cell development, amoebae were washed twice with Volvic mineral water and resuspended in that water at a density of 10^7 cells ml⁻¹. No phosphate, which interferes with the assay for cyclic AMP, was added. The times at which cells became aggregation competent were the same as those which were starved in phosphate buffer (unpublished observations). Cells were starved as agitated suspensions²² and at various times, 5 ml aliquots were added to 0.25 ml 100% TCA and immediately frozen in dry-ice ethanol. The procedure for sample preparation for cyclic AMP assays has been described¹⁹. Assays were performed using the protein kinase kit purchased from Amersham. In these experiments, cells collected from exponential growth ($2-5 \times 10^6$ cells ml⁻¹) consistently became aggregation competent after 5 h of starvation. Therefore, to obtain cells which differentiated after a delay of 12–14 h, we harvested amoebae from early stationary phase cultures (experiment 1). It has been shown that such cells require longer periods of starvation in order to aggregate²⁴ but can be induced to differentiate within 8 h if pulsed with cyclic AMP²⁵. It should be pointed out that the values for cyclic AMP levels produced by cells which become aggregation-competent after 5 h are minimum estimates because of the high levels of phosphodiesterase produced by differentiated cells.

becomes functional in intact cells only after a delay. The following experiments support this hypothesis: Cells which became aggregation-competent within 5 h of starvation showed the periodic changes in light scattering correlated with changes in cyclic AMP synthesis and excretion¹⁸ after 2–2.5 h. In contrast, cells which aggregated after 10–14 h did not show such behaviour during the first 6 h of starvation. Measurements of cyclic AMP produced by these cells showed that they did not synthesise cyclic AMP during the first 6 h of starvation. (Table 1, experiment 1). The levels of cyclic AMP synthesised by cells which were aggregation competent after 5–6 h of starvation are also presented (experiment 2).

Several arguments suggest that applied cyclic AMP pulses stimulate cell differentiation by activating adenylate cyclase rather than by merely replacing the endogenously produced chemotactic signal. Cells which aggregated after prolonged periods of starvation were, when pretreated with cyclic AMP pulses, induced to aggregate and therefore to produce autonomously the chemotactic signal after 6-7 h. An increase in cellular cyclic AMP levels resulting from signal amplification seems to mediate the induction of phosphodiesterase¹⁷ and the repression of the enzyme inhibitor (described here) by cyclic AMP pulses. The stimulatory and inhibitory effects of various compounds on cell differentiation have been correlated with respective increases and decreases in cellular cyclic AMP¹⁸ (P. Brachet and C.K., in preparation). Also Roos and Gerisch have recently shown that a transitory 2-fold stimulation of adenylate cyclase can be elicited by a pulse of cyclic AMP²⁰. It is possible, however, that the activation of adenylate cyclase by external cyclic AMP pulses may not be the sole pathway by which pulses induce cell differentiation.

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Significance of glucose oxidase in lignin degradation

GLUCOSE oxidase (EC 1.1.3.4) is commonly found in the stagnant substratum of lignocellulose degrading fungi accompanying phenol oxidases, cellulases and glucosidases¹⁻⁴, where the steady-state supply of available oxygen is undoubtedly very limited⁵. Yet oxidants other than oxygen have heretofore received little attention as potential co-substrates of this enzyme despite its poor affinity for oxygen^{1,6}. Spectrophotometric studies reported here on the interaction of glucose oxidase with transitory quinone and free-radical lignol oxidation products demonstrate that these intermediates are capable of serving as co-substrates

Table 1 Laccase and glucose oxidase activities in culture extracts of *P. versicolor**

Substrate	Laccase	mU ml ⁻¹ Glucose oxidase
Chlorogenic acid	22-49	—
Glucose	—	68-164

*Extracellular enzyme activity in crude 10,000g supernatant. Cultures were grown on sterile semi-solid rye grass media¹⁶ and cells collected 10-20 d after inoculation. Laccase and glucose oxidase were assayed polarographically¹⁷ at 37 °C on a Gilson Oxygraph equipped with a water-jacketed Clark electrode cell. Routine assays were with 0.1-0.5 ml enzyme in 50 mM potassium acetate buffer, pH 5.0, and 10 µmol of substrate in a total volume of 1.8 ml. Enzyme units are expressed as µmol min⁻¹ of substrate turned over at 37 °C and represent the range seen in assaying five separate colonies of *P. versicolor*.

of the reduced flavoenzyme at low oxygen tensions. Evidence is presented which suggests that glucose oxidase operates in concert with laccase, the principal phenol oxidase of *Polyporus versicolor*^{3,7}, a typical 'white-rot' fungus noted for its capacity to degrade lignin^{1,8-12}, to maintain a laccase-glucose: quinone oxidoreductase cycle in the fungal substratum by which lignol substrates may become highly oxygenated and ultimately degraded.

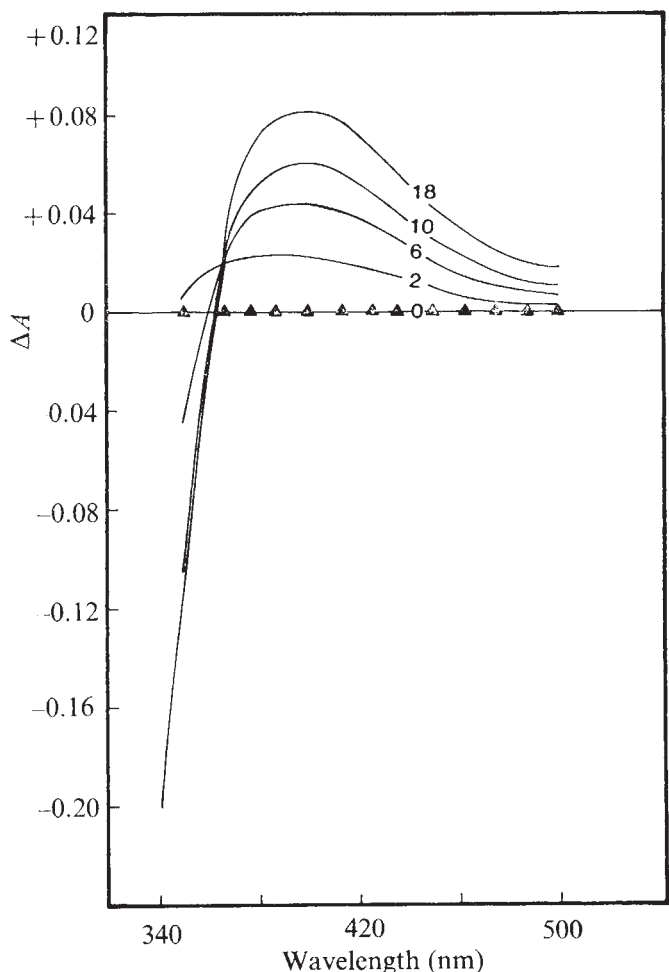


Fig. 1 Generation of quinoid intermediates *in situ* from chlorogenic acid by *P. versicolor* laccase. Sample and reference quartz cells contained 3.0 ml of 0.33 mM chlorogenic acid in 50 mM potassium acetate buffer, pH 5.0. At $t = 0$, 20 µl of stock enzyme (compare with Table 2) was added to the sample cell and the visible spectrum scanned on a Model 11 Carey spectrophotometer. Continuous scans were then made at 2, 6, 10 and 18 min after enzyme addition as indicated. Time-dependent difference spectra were plotted as the difference in absorption between the sample at time, t , following initiation of the reaction, and $t = 0$. —, Difference spectrum seen after 20 min when the same reaction was run with 0.15 mM sodium azide included in the assay mixture.

Five 'white-rot' fungi, *P. versicolor*, *P. abietinus*, *Poria sabicita*, *Phoriatia adiposa* and *Formes fermentarius*, were chosen in initial screening trials for their capacity to produce extracellular phenol oxidases while growing on a semi-solid rye grass substratum supplemented with minimal salt media. Crude enzyme was recovered following filtration and centrifugation of initial slurries of the mycelial straw substrata prepared in 50 mM potassium acetate buffer, pH 5.0. Of the five fungi, *P. versicolor* grew most profusely on the semi-solid medium, and crude enzyme extracts of its substratum were especially rich in laccase and glucose oxidase activity (Table 1).

Dichlorophenol-indophenol (DCIP) was readily oxidised by enzyme mixtures exposed to air, as shown by a sharp blue shift in its adsorption maximum from 575 to 510 nm and a concomitant consumption of dissolved oxygen; it was not metabolised in solutions purged of oxygen and kept under nitrogen (Table 2). Addition of glucose to DCIP-enzyme mixtures under a nitrogen atmosphere brought about complete reduction of DCIP, however, and the readmission of air to these solutions resulted in the reappearance of the DCIP absorption spectrum. Interconversions between DCIP and its dihydro-form were induced through several cycles by appropriate adjustments of the oxygen tension, but continuous exposure of DCIP-enzyme mixtures to atmospheric oxygen resulted in irreversible losses of substrate no longer susceptible to further reductive cycles.

Model quinoid intermediates were also produced *in situ* by mixing laccase from test fungal extracts with the dilignol substrate, chlorogenic acid, as seen by a time-dependent, increased absorption maximum between 380 and 400 nm in the reaction mixture (Fig. 1). Excluding oxygen from the enzyme-substrate mixture, or blocking laccase activity by the addition of azide, prevented formation of this spectrum. These results link the appearance of the 380 nm absorption peak with *in situ* generation of quinoid intermediates.

Figure 2a demonstrates a dual requirement for both glucose oxidase and glucose in bringing about reduction of *in situ*-generated quinoid intermediates. Although most of the quinoid spectrum disappeared with glucose-glucose oxidase additions, there was a gradual accumulation of

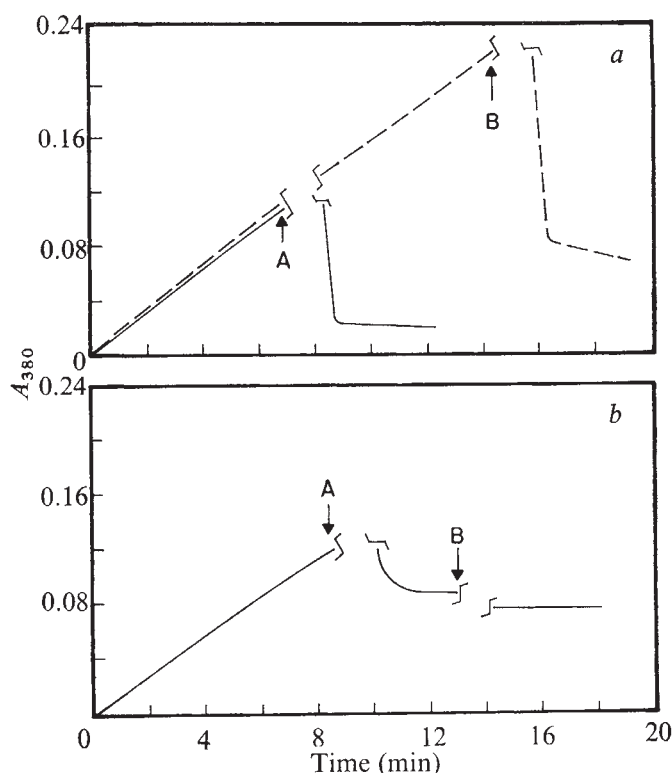


Fig. 2 Effect of glucose-glucose oxidase additions on the reduction of *in situ* generated transitory quinoid intermediates of chlorogenic acid. Quinoid intermediates were generated as described in Fig. 1 and their appearance monitored with time at 380 nm. *a*, Two separate experiments are shown: 3.3 mM glucose was included in the initial reaction mixture of the first experiment (—) and omitted from the second (---). About 7 min after addition of laccase each experiment was briefly interrupted (point A) with addition of 100 μ l of commercial (Sigma) glucose oxidase (120 units). Immediately thereafter the 380 nm absorption was again monitored with time. The experiment lacking glucose failed to register a decrease in 380 nm absorption until glucose (1.0 μ mol) was also introduced to the mixture approximately 8 min thereafter (point B). *b*, The same experiment as in (*a*) was repeated with 3.3 mM glucose in the initial reaction mixture, except 10 μ l (45 μ mol) of sodium azide was added to the sample cell 7 min after initiation of quinoid production by laccase (point A); approximately 6 min thereafter glucose oxidase (120 units) was added to the sample and the 380 nm absorption again monitored as shown.

Table 2 Effect of oxygen and glucose on the laccase-glucose: quinone oxidoreductase cycle

	Substrate	A_{575}	Comments
Experiment 1	DCIP	1.00	Incubated 30 min under nitrogen
		0.56	5 min after admission of air oxidised DCIP products seen in absorption spectrum
Experiment 2	DCIP+glucose	0.00	Incubated 30 min under nitrogen
		1.00	2 min after admission of air absorption spectrum identical with DCIP spectrum.

0.5 ml fungal enzyme extract (approximately 0.3 units laccase; 0.5 units glucose oxidase) and 0.33 μ mol DCIP were mixed together in a final volume of 3.0 ml 50 mM potassium acetate, pH 5.0. Nitrogen was bubbled through the enzyme-substrate mixtures except as noted. The absorbance of DCIP at 575 nm was determined on a Model 11 Carey spectrophotometer; visible spectra of the reaction mixtures were also taken at selected intervals between 375 and 700 nm. Fungal enzyme extracts were prepared by extracting the semi-solid substratum of *P. versicolor* cultures in 50 mM potassium acetate buffer, pH 5.0, filtering the crude slurry through cheese cloth, and centrifugation to yield a cell-free 10,000g supernatant. Stock enzyme was recovered as an ammonium sulphate pellet (0-90% saturation).

material less susceptible to reduction. Azide addition following initial generation of quinoid intermediates blocked further quinoid formation, and a slight decrease in the 380-nm absorption peak occurred thereafter (Fig. 2b). Subsequent additions of glucose and glucose oxidase to the azide treated samples caused only a slight further decrease in absorption. These latter results indicate a rapid turnover of quinoid intermediates. The residual, non-reduceable background absorption at 380 nm seems to coincide with a shift in the average absorption spectrum towards longer wave lengths (compare with Fig. 1) and probably reflects concomitant and irreversible structural rearrangements among transition state intermediates (for example, quinoid condensates, polymerisations) (ref. 13).

The enzymatic oxidation of glucose by glucose oxidase is known to occur in two steps: (1) oxidation of glucose to gluconolactone through the transfer of two electrons to a flavin prosthetic group on the enzyme; and (2) reoxidation of the reduced flavoenzyme through a second two electron transfer to a suitable electron acceptor^{4,14}, and thus the capacity of oxidants to participate in the enzymatic oxidation of glucose rests in their ability to accept electrons from the reduced enzyme. It is noteworthy that the K_m of glucose for glucose oxidase decreases from 4.2 to 1.9 μ M upon decreasing the oxygen tension in the gas phase from

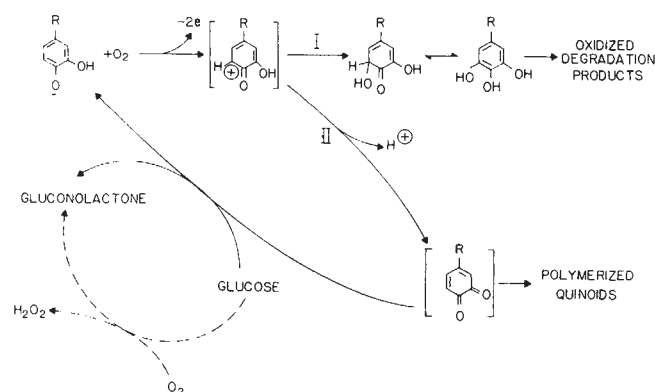


Fig. 3 Schematic flow diagram of postulated laccase-glucose:quinone oxidoreductase cycle and its significance in the degradation of lignol substrates.

20 to 5%, respectively, but that glucose oxidation is less than half $V_{\max}$ at atmospheric oxygen tensions in the presence of saturating concentrations of glucose^{4,6,15}. Oxygen is thus a poor substrate of glucose oxidase, whereas glucose is an excellent substrate (for example, reductant) whether oxygen is present or absent from solution. Moreover, at physiologically low oxygen tensions the ability of quinone or free-radical lignol intermediates to compete with oxygen as oxidants of glucose oxidase would be expected to be of greater significance because of an improved ratio of quinoid concentration over that of oxygen. The experimental results of this study corroborate these observations.

Figure 3 outlines a metabolic role for glucose oxidase which may provide a rationale for laccase-glucose:quinone oxidoreductase cycling. Initial abstraction of electrons from a lignol substrate by laccase transforms the substrate into an electron deficient transition state intermediate, which may undergo nucleophilic attack by hydroxylation as illustrated by route I of Fig. 3. On the other hand, an electron shift and loss of proton may occur to yield a transitory quinone intermediate as shown by route II of Fig. 3. Without glucose:quinone oxidoreductase activity, transition state intermediates produced by laccase would accumulate, condensing and polymerising with products secreted into the microbial substratum. Glucose oxidase may thus serve two functions in the substratum: (1) to protect the metabolic processes of the microbial cell from toxic accumulations of quinoid intermediates; and (2) to greatly improve the efficiency of lignol oxidations by channeling substrates through productive degradative routes.

In view of these observations, the physiological significance of oxygen as a co-substrate of glucose oxidase is worth re-examining, possibly as a potential modifier of glucose:quinone oxidoreductase activity, because of its capacity to compete as an oxidant of glucose oxidase, and in the process to uncouple normal channeling of lignol oxidation products through the laccase-glucose:quinone oxidoreductase cycle.

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Unusual mitosis in the red alga *Porphyridium purpureum*

Porphyridium purpureum (Bory) Drew *et* Ross (Syn.: *P. cruentum* Nägeli) is a classical laboratory organism and one of the few red algae easily grown in large quantities. This would make it an obvious candidate for investigation of red algal mitosis with the electron microscope. The nuclei are, however, quite small and fast dividing, so that previous attempts remained apparently unsuccessful^{1,2} and left the much more complex species *Membranoptera platyphylla* the only red alga where mitosis had been satisfactorily described³. We are now able to report that mitosis in *Porphyridium* is not only quite distinct from that in *Membranoptera* but also presents a unique association of microbodies with the spindle poles.

In order to increase the chance of observing mitotic figures, a culture of *P. purpureum* strain 161 of the University of Indiana culture collection grown in artificial seawater⁴ was synchronised by a 12 h light-12 h darkness cycle according to Gense *et al.*⁵ modified by Knappe⁶. Cells collected the fourth day after the beginning of synchronisation, 8 h after the beginning of the light period (the best time for mitosis as estimated from cell count curves and observation of feulgen-stained material), were fixed with glutaraldehyde, postfixed with osmium, sectioned and stained with uranyl acetate and lead.

Despite the fact that several hundred cells were examined,

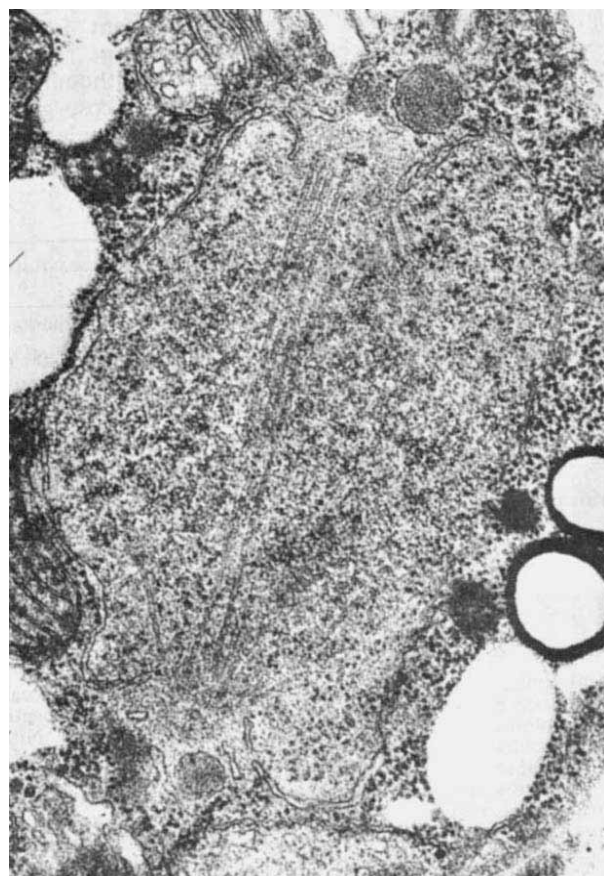


Fig. 1 Metaphase ($\times 16,750$).

only four were in metaphase and for only one did the plane of the section include both spindle poles (Fig. 1). The nuclear envelope is well preserved except for large polar fenestrae where microtubules end in a ribosome-free area delimited by fragments of endoplasmic reticulum and microbodies (one to three). No well defined pole body is apparent and the microtubules seem to be divided into a central bundle of interpolar ones and peripheral ones that end in small darker masses that might be badly condensed chromatin without kinetochores. The nucleolus is apparently dispersed, with some ribosome-like particles visible in the periphery of the nucleus.

A few tangential sections of nuclei that might be in prophase suggest that at that time an amorphous microtubule-organising centre might be present, to which extranuclear microtubules are connected (Fig. 2). This is however insufficient to demonstrate an extranuclear origin of the spindle.

Nuclear division and rebuilding of the nucleolus are performed before the onset of cytokinesis.

Microfilaments about 10 nm in diameter similar to those reported in other algal and fungal nuclei⁷⁻⁹ have also been observed, but not in dividing nuclei (Fig. 3).

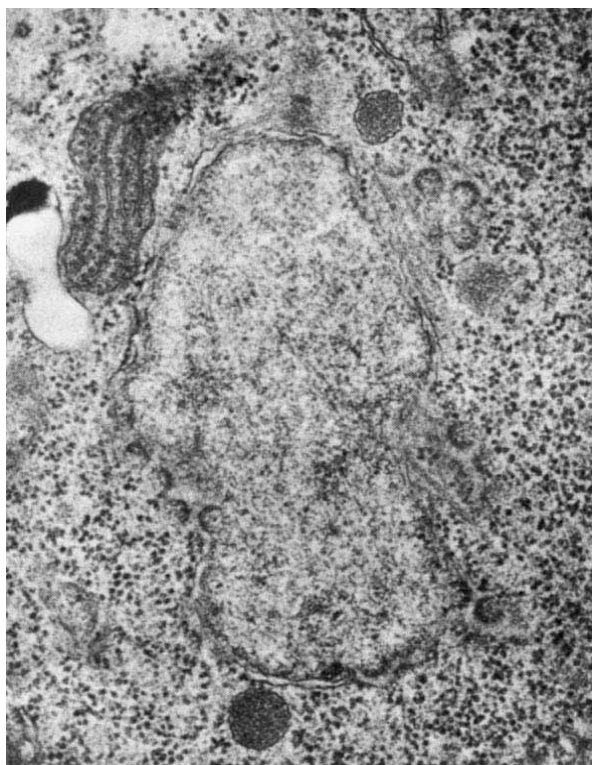


Fig. 2 Prophase (?); tangential section of the nucleus ($\times 16,750$).

It is notable that we have observed none of the figures previously interpreted as cytoplasmic invaginations in the nucleus during mitosis¹, although we have worked with the same strain. From further studies by those authors² it seems that those figures were attributable to a viral infection that did not manifest itself in our culture.

The reported nuclear behaviour of *Porphyridium* is quite distinct from that of the more complex red alga previously studied³. It lacks the polar rings and kinetochores (unless those structures have escaped our sections), the well condensed chromosomes and the doubling of the nuclear envelope by endoplasmic reticulum. The polar microbodies (a feature verified in three other nuclei) are as far as we know a unique feature. A microbody is pictured by Franke and Reau¹⁰ near a dividing nucleus of *Phycomyces*, but other pictures seem to imply that this is not a regular feature. In *Pyramimonas*¹¹, a single large microbody has a peculiar behaviour during mitosis but might be more associated with the flagellar bases than to the nucleus.

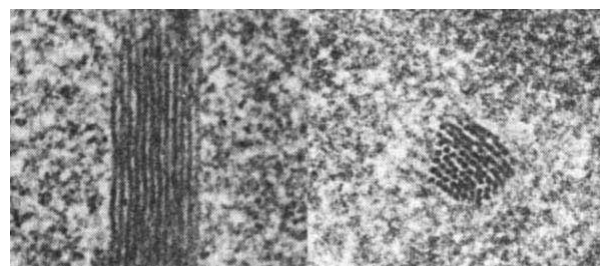


Fig. 3 Microfilaments in the nucleus: left, longitudinal section; right, transverse section ($\times 33,500$).

The microbodies are apparently similar to those previously studied in *Porphyridium* by Oakley and Dodge¹².

The large polar fenestra is rather similar to that of the Basidiomycetes *Poria latemarginata*¹³ and the lack of condensed chromosomes is a typical feature of higher fungi but at the moment *Porphyridium* cannot be clearly related to any other organism on the basis of its mitosis¹⁴⁻¹⁶.

Its mitosis is anyway not closer to that of Cryptophyceae¹⁷ than that of *Membranoptera* and so does not change the situation in the controversy on possible relationships between Rhodophyceae and Cryptophyceae¹⁸⁻¹⁹.

Studies of further red algae, especially the primitive ones would obviously be extremely interesting as the mitotic type of the group cannot be only characterised by what is known in *Membranoptera*.

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Carotenoid transformations during chloroplast development in *Scenedesmus obliquus* PG1 demonstrated by deuterium labelling

THE unicellular green alga *Scenedesmus obliquus* (Chlorophyta), when grown photosynthetically, contains pigments (chlorophylls and carotenoids) similar to those of the chloroplasts of higher plants¹. Several mutant strains¹ have abnormal pigmentation¹. One of them, PG1, when grown in the dark accumulates the acyclic carotenoids phytoene (7,8,11,12,7',8',11',12'-octahydro- ψ,ψ -carotene), phytofluene (7,8,11,12,7',8'-hexahydro- ψ,ψ -carotene) and ζ -carotene (7,8,7',8'-tetrahydro- ψ,ψ -carotene) in place of cyclic carotenes and xanthophylls, and cannot make more than trace amounts of chlorophylls. The pigmentation of light-grown PG1, however, is virtually identical to that of the wild type. When dark-grown PG1 is illuminated,

the content of acyclic carotenoids decreases and that of cyclic carotenoids increases². Time-course studies³ agree with the hypothesis that the cyclic carotenoids produced on illumination are formed from the acyclic precursors that accumulate in dark-grown cultures. It has not been possible to confirm this by radioisotopic labelling, but we have now done so using deuterium labelling. Our procedure also provides a general method for following the kinetics of formation of important chloroplast constituents during chloroplast development in this and other organisms.

Wild-type *S. obliquus* can be cultured (autotrophically) in deuterated medium (99+ % D₂O; ref. 4). By culturing *S. obliquus* PG1 in the dark on ordinary (H₂O) medium and then transferring to D₂O and illuminating we have developed a method in which deuterium labelling is used to examine changes that occur in the carotenoid composition of the organism during illumination. *S. obliquus* strain PG1 was grown heterotrophically in the dark, in which conditions it accumulated ζ -carotene, phytoene and phytofluene as its main carotenoids¹. The cells were harvested, resuspended in D₂O without added nutrients, and starved overnight by shaking in the dark to deplete starch reserves. A mixture of air-CO₂ (95:5) was then bubbled through while the cell suspension was illuminated for 4 h. The main cyclic carotenoids, α -carotene (β,ϵ -carotene), β -carotene (β,β -carotene), lutein (β,ϵ -carotene-3,3'-diol) and zeaxanthin (β,β -carotene-3,3'-diol) were subsequently extracted and purified¹ and examined by mass spectrometry. The important features of the mass spectrum of β -carotene are illustrated in Fig. 1 and discussed below; similar results were obtained for the other carotenoids. Three features in the mass spectra are particularly important.

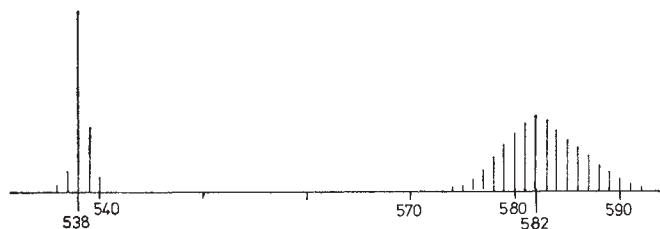


Fig. 1 Features of the high mass region of the mass spectrum of deuterio- β -carotene produced by *S. obliquus* PG1 grown in the dark and then resuspended in D₂O and illuminated.

(1) There was virtually no trace of the normal molecular ion of α -carotene or β -carotene (both C₄₀H₅₆) at m/e 536 or of lutein or zeaxanthin (both C₄₀H₅₆O₂) at m/e 568. There had therefore been virtually no synthesis of undeuterated cyclic carotenoids. (2) In all cases by far the most intense ion in the high mass region corresponded to m/e (M+2) in the spectrum of the unlabelled carotenoid, that is at m/e 538 for the carotenes and 570 for the xanthophylls. This indicated that the most abundant molecular species in each case was one containing two deuterium atoms. The mass spectrum of recovered, uncyclised ζ -carotene showed only the normal molecular ion at m/e 540 (C₄₀H₆₀) with no ion at m/e 542 corresponding to a dideuterio species. (3) The group of ions at approximately m/e 574–592 in the spectra of α - and β -carotenes and at approximately m/e 606–622 in the spectra of lutein and zeaxanthin, in each case accompanied by characteristic (M–deuterotoluene) fragmentations, indicated the presence of molecular species containing extensive deuterium labelling (average composition C₄₀H₁₀D₄₈ for carotenes and C₄₀H₁₂D₄₄O₂ for the xanthophylls). The relative intensities of these ion groups gave a measure (assuming similar volatilities for all the molecular species in the mass spectrometer ion source) of the extent of new synthesis of cyclic carotenoids from small molecules, for example CO₂ and D₂O occurring during illumination.

The cyclisation reaction of carotenoid biosynthesis (Fig. 2) is generally considered to be a proton-initiated process⁵. Thus if an acyclic precursor carotenoid is formed in ordinary (H₂O)

medium and then cyclised in D₂O, one deuterium atom will be introduced at C-2 during each cyclisation, that is, bicyclic carotenoids will contain two deuterium atoms. The mass spectrometric assay therefore directly demonstrates that in greening *S. obliquus* PG1, α - and β -carotenes, lutein and zeaxanthin are all formed by cyclisation of existing acyclic precursors (for example ζ -carotene, presumably first desaturated to lycopene). Conclusions drawn previously³ from the results of kinetic studies without isotopic labelling are therefore valid.

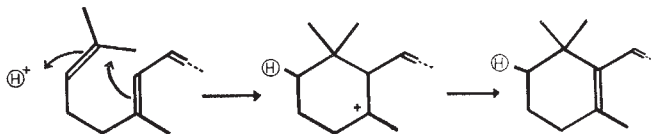


Fig. 2 Mechanism of the cyclisation reaction of carotenoid biosynthesis.

Perhaps the most important feature of this procedure is that, by mass spectrometric assay, molecules synthesised from pre-formed intermediates can be distinguished from molecules formed by new synthesis. Studies of the time-course of events occurring during chloroplast development are therefore possible. For example, when dark-grown PG1 is transferred to D₂O and illuminated, and aliquots of the greening cells are removed after various time intervals and the cyclic carotenoids are extracted and assayed by mass spectrometry, it is apparent that in the early stages of greening (up to 2 h) the cyclic carotenoids are derived mainly from the preformed cyclic intermediates, whereas after longer times the cyclic carotenoids are increasingly being produced by new synthesis. Note, however, that the greening process occurs much more slowly in D₂O than in H₂O.

This procedure clearly has potential as a general method for studying metabolic pathways or developmental processes that can be interrupted, for example by inhibition, mutation, lack of light energy, but can then be completed under appropriate conditions. If the first phase of the process (up to the interruption) takes place in ordinary (H₂O) medium and the second phase (after removal of the interruption) is allowed to proceed in D₂O or D₂O-based medium, then mass spectrometric assay of the type described above can be used to differentiate between compounds produced entirely during the first phase, entirely during the second phase, or, in appropriate cases such as cyclic carotenoids, formed during the second phase from intermediates produced during the first phase. The method can thus be used to obtain information about the origins of various chloroplast components, the time-course of their formation and their relative importance in the chloroplast structure, during development of algal chloroplasts. The method is also applicable to studies of the development of the photosynthetic apparatus in photosynthetic bacteria. We have used a related procedure to demonstrate the formation of cyclic carotenoids from accumulated lycopene in a *Flavobacterium* species and to determine the stereochemistry of the proton-initiated cyclisation reaction. (G. B., W. J. S. L., N. J. Patel and T. W. G., unpublished).

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reviews

Curious little book on rabies

George M. Baer

Rabies: The Facts. Edited by Colin Kaplan. Pp. 116. (Oxford University: Oxford and London; Corgi: London, 1977.) Hardback (OUP) £1.95 paper-back (Corgi) 75 pence.

THIS is a curious little book on rabies. If it is intended for a scientific audience, it is the first such book I know of without references. If it is a book for the general public, it contains highly specialised information on nucleoproteins, structural proteins of the virus, and comments rather advanced for the average reader such as: "In their model of rabies virus, Vernon and his colleagues have depicted the larger of the two M proteins (M_1) as being adjacent to the ribonucleoprotein".

The book is divided into seven chapters, beginning with a discussion of the world problem, and emphasising the under-reporting of the disease. Under-reporting has also been discussed in a notable 1967 report by Sanmartín *et al.* (Sanmartín, Correa, Dueñas & Muñoz, *Memorias del Primer Seminario Nacional Sobre Rabia*, Medellín, Colombia, 155–161, 1967) in which 1.7% of 1,596 autopsies in the University Hospital in Cali, Colombia, showed that death was due to rabies.

It is unfortunate that no maps or tables are included in a discussion of world rabies epidemiology, and that the description of the fluorescent antibody technique is not accompanied by a diagram making it a little more intelligible for the layman. The technique has been of tremendous value in rabies diagnosis and investigations.

The descriptions of the disease attest to the terror that rabid dogs once instilled in the hearts of English countrymen, much as it does today in thousands of people in most developing countries. It has often been noted that rabies infection in the field frequently results in antibody production in a sizeable percentage of animals, much as described in this book. This includes animals such as insectivorous bats (Burns & Farinacci *J. Infect. Dis.* **97**, 211; 1955), mongooses (Everard, Baer & James *J. Wildlife Dis.* **10**, 190; 1974), dogs (Doege & Northrop *Lancet*, **ii**, 826;

1974) and other fairly resistant species. Much is made of the two human recoveries from rabies (Hattwick *et al. Ann. Intern. Med.* **76**, 931; 1972; Porras *et al. Ann. Intern. Med.* **85**, 44; 1976), as well as one purported carrier dog (Veeraraghavan *et al., Ann. Rep. Pasteur Institute of Southern India*, 66, 1968), yet it has not been pointed out that these are bizarre exceptions to the rule.

The chapter on rabies virus itself by Crick and Brown is a complete description of the morphology, chemistry and structural subunits of the virus, and of rabies-related viruses. It also covers replication of the virus, synthesis of virus components and other products from infected cells.

The chapter on human rabies includes various aspects of the disease in man, including susceptibility, symptomatology, diagnosis and prevention. An amazing anecdotal series of cases of rabies transmitted from man to man is succeeded by a statement that "... there is no completely documented case of man transmitting rabies to man", in which case it seems pointless to even mention the dramatic anecdotal cases. Another amazing statement is: "For reasons which are unknown rabies is up to seven times commoner in men than in women: this also applies to animals". This statement may be applicable to man (see review by Hattwick in *The Natural History of Rabies*, Academic, New York, 1975), but it is certainly not for animals. Verts (*The Biology of the Striped Skunk*, University of Illinois Press, Urbana, Chicago and London, 1967) writes that "Of the 20 rabid striped skunks caught in northwestern Illinois, 19 (95.0 per cent) were females". This type of information should not be included unless it is properly documented.

The chapter on rabies in animals includes a table listing incubation periods for various species, with errors: for example, incubation periods of more than 5 months have been reported for foxes (Sikes, *Am. J. vet. Res.* **23**, 1041; 1962) and periods of more than one year have been reported in cattle (Abelseth & Lawson, cited by Bell, *The Natural History of Rabies*,

Academic, New York, 1975). Photographs of humans and animals with rabies, as well as electron micrographs of rabies virus are included in this chapter. There is rightful emphasis on the variability of the signs in dogs, and on the fact that relatively few develop furious rabies. It is again unfortunate that no references are included; for example, I am not acquainted with the outbreak of rabies reported in pigs. Other curious statements such as "Deer are as prone to rabies as cattle..." should be documented. The chapter ends with the astounding suggestion that "There is a potential danger here (that is, Britain) that rabies might enter this country and become enzootic in mink much the same way it has become established in the mongoose..." One wonders why this has not already occurred in the numerous countries where rabies is enzootic and mink are plentiful.

The next two chapters are on fox behavioural ecology and the prospects of wildlife rabies in Britain. They include a description of fox behaviour and rabies in the fox, with summaries of studies done in Britain and other countries. These chapters are basically well presented and include much useful information. The possible transmission of rabies from cats to foxes is emphasised, without any explanation as to why this mode of transmission is believed to occur. In fact, Wachendörfer has shown (*Dt. tierärztl. Wschr.* **69**, 555; 1962) that the case of rabies transmitted from foxes to cats are closely related and the two epizootiological curves can be virtually superimposed.

The short chapter on rabies vaccines by Turner encompasses most of what is known today about human and veterinary vaccines, with emphasis on the difference between pre-exposure vaccination (such as vaccination of animals and of people at risk to exposure in the laboratory), and post-exposure vaccination. □

George M. Baer is Chief of the Viral Zoonoses Branch of the Center for Disease Control (Lawrenceville Facility), Lawrenceville, Georgia.

European wildfowl

Wildfowl of Europe. By Myrfin Owen. Colour plates by Hilary Burn and foreword by Sir Peter Scott. Pp. 256. (Macmillan: London and Basingstoke, 1977.) £15.

QUITE often popular books of this sort are written to support the illustrations and this may have been the original idea for this volume. The author admits that this book does not pretend to summarise all known information about wildfowl. He attempts to cover most aspects of the birds' lives unclouded by tedious detail and he confesses to a personal bias in what he has omitted. The first quarter of the book deals with taxonomy, waterfowl movement, populations dynamics, migration and man's relation to waterfowl. Helped as he has been by the scientific staff of the Wildfowl Trust, the author has included some new quantitative information in these chapters. The final chapter of this section is spoiled by his unnecessarily chauvinistic attitude, which gives the reader the misleading impression that one can only see wildfowl in three localities in Britain—three reserves administered by his own organisation.

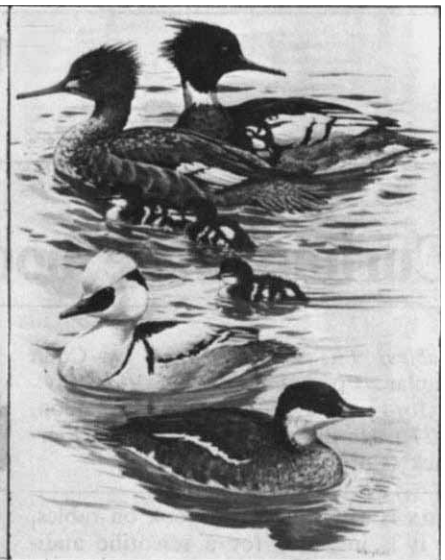
The remaining three-quarters of the book provides a brief history of all the species of wildfowl recorded in Europe. Many chapters contain new information resulting from research undertaken by the Wildfowl Trust: particularly valuable are the figures for European populations based on counts organised



Barnacle Goose

by the International Waterfowl Research Bureau, and the accounts of the feeding ecology of nearly all the main species of duck. But there is little about breeding behaviour in the individual accounts. Four useful appendices summarise information about nests, eggs and young of wildfowl, winter weights and measurements, winter food and waterfowl in captivity.

This volume is illustrated by coloured plates—for which incidentally there is no index or list—by Hilary Burn, a promising young bird painter whose work has developed rapidly. But because of her predilection for the use of black to create shadows, many of the plates of the colourful species such as wigeon, pochard and mallard are sadly lacking in natural sparkle. The



Red-breasted Merganser (above) and Snipe (below)

design of some of the plates could show more imagination and the scale is sometimes awry. Joe Blossom's line drawings are generally competent at underlining points made in the text.

Interesting though it is, I find it difficult to accept that this rather popular volume is really worth the £15 charged for it: while the wildfowl enthusiasts will buy it, the specialist would have expected something much more comprehensive. Costs might have been less if the margin had not been so wide—75 millimetres for a single page or 150 millimetres when the book is open.

Peter Conder

Peter Conder recently retired as Director of the Royal Society for the Protection of Birds.

Push-button biology

Human Biology: An Exhibition of Ourselves in the new Hall of Human Biology at the British Museum (Natural History), London. A permanent exhibition, admission free.

Human Biology: An Exhibition of Ourselves. Pp. 120. (British Museum (Natural History) and Cambridge University: London, 1977.) Hardback £5.00; paperback £1.95.

THE dinosaurs and whales, and halls of decorously deceased representatives of the animal kingdom, at the British Museum (Natural History) (BM (NH)) have been joined by an exhibition which requires more of the visitor than tireless legs and unglazable eyes. The Hall of Human Biology is the first fruit of the major reorganisation which is intended, during the next 30 years or so, completely to rearrange the museum's specimens. They have been presented for more than 100 years according to Victorian notions of the plan of the Creator, which are no longer deemed

appropriate. In future visitors will be expected to discover as well as to learn, and the specimens will be accompanied by buttons to push, handles to turn and games to play. Although in Britain this represents a revolutionary new concept in museums, it has been flourishing abroad for some time in such centres as the Oregon Museum of Science and Industry in Portland.

In *An Exhibition of Ourselves*, which fills the Hall of Human Biology, visual, tactile and olfactory aids can be manipulated to reveal details of growth and development, and of the control of bodily functions and their responses to the external environment. Much of the material has been incorporated into the accompanying book, which consequently tells its story as much through pictures as through the text. In the tradition of such BM (NH) publications, the book can be used independently, although it follows closely the plan of the exhibition.

Willing learners, young or old, with some experience of biology textbooks should find that the exhibition usefully

and enjoyably complements their knowledge. For them animated models will explain homeostasis and neuromuscular control; a slide show will clarify the events of gestation and birth, and an optical illusion will tell something about perception. Afterwards *Human Biology* will remind them of the concepts they have encountered.

Visitors with little or no acquaintance with biology, however, may have to concentrate quite hard on the exhibition. And that could be difficult when the maze of exhibits is swarming with exuberant youth apparently determined to flash all lights and ring all bells at once. But a subsequent session with the book will probably be very helpful for visitors who cannot absorb all the information offered by the exhibits.

The museum authorities foresee a need to control the flow of visitors through the exhibition, and they are waiting to see how it survives the rigours of the next few months.

Mary Lindley

Mary Lindley is Assistant Editor of Nature.

Life as a self-perpetuating information system

Genetic Evolution. By Chen Kang Chai. Pp. 341. (University of Chicago: Chicago and London, 1976.) £15.

THE theme of this work is that life is a self-perpetuating information system and that at all levels this undergoes revision or evolution. The author considers, in sequence, the evolution of prebiotic molecules, of genes, symbiosis, the evolution of chromosomes, of biological structure and function and of populations. In spite of protestations otherwise, this cannot be considered a text book; indeed its use as such would be irresponsible. Those without a broad elementary understanding of modern genetics would find this book difficult, and it contains much that should not be uncritically accepted by a genetics undergraduate. It is a hybrid between a collection of essays and a textbook (say, of population genetics); the phenotype of the organism is stimulating and

interesting in places; biased and superficial at others. The subjects covered are so broad that response to a particular chapter is likely to be conditioned by previous experience of its subject matter.

Chapter 1 is concerned with the evolution of biological molecules and the genetic apparatus. It is straightforward and recalls the work of Oparin, Calvin and others on the evolution of organic molecules from inorganic precursors. The next chapter deals with the evolution of genes and perpetuates the belief that the rate of amino acid substitution (and thus the rate of change of DNA) is constant for particular proteins. This is no longer upheld by data for cytochrome C and haemoglobin. The α and β chains of haemoglobin have evolved at different rates, and the same chain has evolved at different rates during vertebrate history.

The third and fourth chapters deal with symbiosis as a precursor to sexual reproduction and with the evolution of eukaryote chromosomes respectively. These two chapters are absorbing but one is left with the uncomfortable feeling that the evidence

for each thesis is tenuous to the extreme. When dealing with the evolution of structure and function the book is more convincing and the (freely admitted) sin here is one of omission. The remaining chapters largely deal with population genetics with the material arranged to suit the underlying theme of the book. Here, the discussion of genetic load seems uncritical as is the examination of the current controversy between the neo-Darwinists and those subscribing to neutral mutation theory. The author obviously believes that most variability is of selective importance; yet as with data on the constancy of the rate of amino acid substitution, he does not stress alternative arguments.

If one accepts this book's F_1 status (and protects the innocent), it is interesting and enjoyable. With appropriate supplementary material it should form the basis of many lively honours level seminars and discussions.

J. A. Bishop

J. A. Bishop is Senior Lecturer in the Department of Genetics at the University of Liverpool.

Chemical lasers

Handbook of Chemical Lasers. Edited by R. W. F. Gross and J. F. Bott. (Wiley Interscience: New York and London, December, 1976.) Pp. x+744. £31; \$49.

THE preface of this volume describes how a meeting was convened in California in September 1964 to discuss the possibility of nonequilibrium chemical excitation and pumping of lasers, and how by the close of the conference a young graduate student was able to claim that he had demonstrated the first photo dissociation laser. In that experiment excited iodine atoms were formed by the photoinduced rupture of alkyl iodine bonds; this work led naturally to the demonstration of chemically-pumped emission in photolytically-initiated hydrogen-chlorine explosions by the same workers (Kasper and Pimentel) in 1965. It is instructive to speculate why so few of the resultant publications in this interdisciplinary field have emanated from the United Kingdom or indeed why relatively few of the twenty authoritative contributors to this handbook work in western European laboratories.

The editors do not claim to have

created a basic text book, but rather an up-to-date compendium of relevant (high gain) laser physics, the gas dynamics of reactive flows and the chemical kinetics of nonequilibrium reactions. Pulsed and continuous hydrogen-halide lasers, the carbon monoxide laser, high power photochemical iodine lasers and metal-atom oxidation lasers are treated in detail; between 40 and 240 references per chapter ensure that most of the published journal and report literature between 1967 and late 1974 is well represented. Problems of numerical (gain) modelling certainly merited (and received) a chapter of their own; rather unexpectedly the treatment of optical resonators also extended to some 120 pages. Like many of the other contributions, however, this chapter (by Chester and Chodzko) was a delight to read; their treatment of stable and unstable resonator theory, and the complications introduced by the laser-gain medium in matching theory to experiment, is a jewel of conciseness. The book is well produced and edited, a 'must' for libraries, but rather expensive for the individual research worker.

Ian Spalding

Ian Spalding works on laser applications at the UKAEA Culham Laboratory, Abingdon, UK.

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Explanations in criminology

Behaviour and Misbehaviour: Explanations and Non-Explanations. By Nigel Walker. Pp. ix+154 (Blackwell: Oxford, 1977.) £5.

MOST new disciplines go through a period of introspection in which they examine the philosophical status of their theories and the adequacy of their methods. Criminology has not yet emerged from this stage, and in *Behaviour and Misbehaviour* Professor Walker examines the nature of explanation in that subject.

He distinguishes between "necessity explanations" and "possibility explanations". The former are used to explain regular events such as the daily rising of the Sun or the expansion of a gas on heating, the latter to explain exceptional or unique events. If an iceberg appears as far south as Rockall, we do not ask why it was *necessarily* there but what made it *possible* for it to be there: the answer required is a narrative involving such factors as a particularly hard winter in Greenland, the prevailing ocean currents, and the existence of strong north-westerly winds. Professor Walker calls the former type of explanation "scientific" and the latter "narrative". He then argues that because much criminal behaviour is exceptional, criminologists may have to be satisfied with narrative rather than scientific explanations. He is probably right: even rates of crime, as well as individual crimes, are determined by many interacting features some of which may be unique to a given society. But it is not clear that the difference between theories in the physical and social sciences is entirely captured by the distinction between "narrative" and "scientific" explanations: as Professor Walker acknowledges, even the physical scientist usually has to fall back on the narrative explanation to account for individual events such as the failure of a bridge.

A further distinction drawn by Professor Walker is that between explanation by analogy and explanation in terms of mechanisms. The difference is by no means clear-cut: an explanation by analogy may in fact be implicitly proposing a mechanism the details of which are not fully worked out. When Harvey had the insight that the heart was a pump was he suggesting an analogy or a mechanism? Exception can be taken to Walker's contention that explaining human behaviour by computer simulation is only giving an analogy: a computer program can rep-

resent the mechanisms underlying behaviour in just the same way as a set of equations can represent the mechanisms underlying the behaviour of a physical system.

Some of Professor Walker's omissions are suprising: in discussing scientific explanations he says little about formal rigour, simplicity, elegance or generality. He does, however, rightly castigate his fellow criminologists for the idiocy of some of their disputations such as the controversy over whether a given phenomenon can only have a single explanation and the dispute about the range of phenomena that fall within the province of criminology. He also gives short shrift to the arguments

of Peters, Winch and others that human behaviour can never be understood in terms of causes.

I have two complaints about the book. For its exorbitant price, one might reasonably have expected the publishers to have hired a more conscientious proof reader. The second complaint is more unusual: although the book raises interesting issues, it is too short to do justice to the complexity of its subject matter, and it should have been several times as long.

Stuart Sutherland

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition at the University of Sussex, UK.

Enzyme kinetics

Enzyme Kinetics. By D. V. Roberts. Pp. 326. (Cambridge University: Cambridge, London, New York and Melbourne, 1977.) Hardback £15; paperback £5.50.

THIS book deals with the use of mathematical expressions to describe the behaviour of enzymes and enzyme systems. Although the author works through the derivations of many of the equations that he presents, some familiarity with the use of determinants and the calculus is necessary if the reader is to follow the arguments used, and the book could not be recommend to mathematically weak students. For those who are not frightened-off by the mathematical treatments used, the author's comparison between the use of determinants and the schematic King-Altman procedure for deriving initial-rate equations is valuable, as is his treatment of the use of the Laplace-Carson operator method for solving the differential equations describing pre-steady-state behaviour. At times the derivation of equations seems to take precedence over consideration of the phenomena that they attempt to describe. In the chapter on allosteric proteins, for example, it is unfortunate that the author does not give more emphasis to the predictions made by the different models that he considers and to ways in which they may be distinguished.

The coverage given to different aspects of enzyme kinetics varies greatly and some topics, such as the effects of pH and temperature on enzyme activity, are given rather shallow treatments. In some places the

analyses presented are misleading. The section on the use of product inhibition as an aid to defining kinetic mechanism, for example, fails to consider the effects of abortive ternary complex formation in random mechanisms and thus predicts an inhibition pattern for the rapid-equilibrium case which is in fact rare. In addition failure to distinguish clearly between the exclusive and non-exclusive cases of the concerted model of co-operativity leads to confusion in the analysis of the curve shapes predicted by this system. The author's differentiation between the steady-state and rapid equilibrium treatments of kinetic mechanisms is also confused and this is particularly evident in his treatment of non-competitive inhibition.

The main strengths of this book lie in its treatments of the kinetics of coupled enzyme systems and of computer simulation methods. This latter chapter, which considers the use of both analogue and digital computers, is particularly valuable since this important topic is usually ignored in books of this type. The author's enthusiastic treatment should capture the interest of many students and although the section on digital methods would not be sufficient to enable the reader to write his own simulation program, it provides an excellent introduction to the terminology and methods used. The sections on the use of analogue computers define the components used and give sample programs for simple kinetic systems. These programs should be sufficient to stimulate many enzymologists with access to an analogue computer to take this approach further.

K. F. Tipton

K. F. Tipton is Lecturer in Biochemistry at the University of Cambridge, UK.

newly on the market

These descriptions are prepared by the staff of *Nature* on the basis of material provided by manufacturers. The Reader Enquiry Coupon faces the inside back cover.

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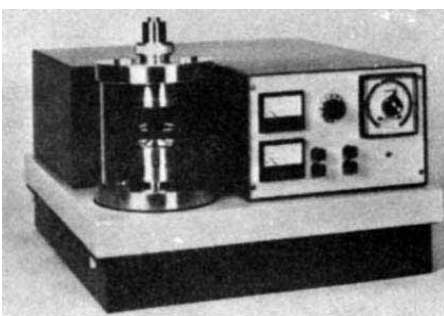
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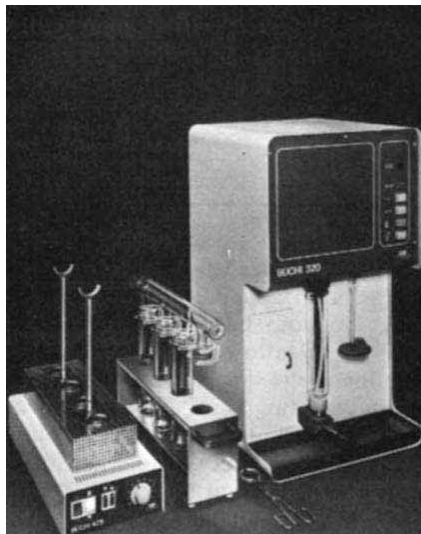
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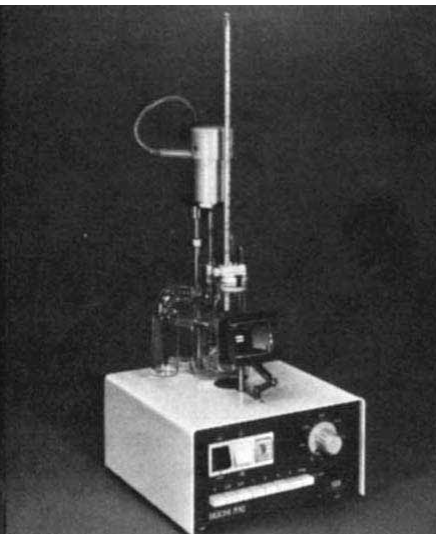
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Büchi melting point apparatus

Cell harvester with pre-programmable four wash sequence. Dynatech. For techniques where it is desirable to harvest cell cultures with more than one wash fluid, the Multiwash has been designed to deliver, in sequence, up to 4 wash fluids, including TCA, completely automatically to a row of 12 wells of a Microtiter plate, while simultaneously aspirating the contents of each well onto a fibre glass filter for subsequent analysis. Simple push button control enables selection of any combination of wash fluid; and wash times for each fluid may be independently varied between 2 and 60 s by pre-programmable time controls. Water vacuum may be sufficient for operation but a suitable vacuum pump is an option, if mains pressure is insufficient. Also standard inter-changeable wash/aspirate heads to facilitate harvesting from standard test tube racks and also have the facility to manufacture special heads to customer specification, if desired.

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The educational version of the K-20 provides additional measuring devices which allow students to carry out complete evaluations and assessments of various cryogenic phenomena.

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